(12)特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局

(43) 国際公開日
2002年1月17日 (17.01.2002)

PCT

(10) 国際公開番号
WO 02/04626 A1

(51) 国際特許分類⁷: C12N 15/09, C07K 14/37, 16/14, G01N 33/15, 33/50, A61K 39/395, 45/00, 31/4355, 31/4365, 31/437, 31/44, 31/472, 31/4725, 31/5377, A61P 31/10, C07D 213/16, 213/61, 213/65, 213/69, 213/74, 217/18, 217/20, 401/10, 401/12, 405/06, 405/10, 405/12, 471/04, 491/048, 491/056, 495/04, 498/04, 513/04

(21) 国際出願番号: PCT/JP01/05899

(22) 国際出願日: 2001年7月6日 (06.07.2001)

(25) 国際出願の言語: 日本語

(26) 国際公開の言語: 日本語

(30) 優先権データ:
特願2000-206968 2000年7月7日 (07.07.2000) JP
特願2000-316027 2000年10月17日 (17.10.2000) JP

(71) 出願人 (米国を除く全ての指定国について): エーザイ株式会社 (EISAI CO., LTD.) [JP/JP]; 〒112-8088 東京都文京区小石川4丁目6番10号 Tokyo (JP).

(72) 発明者; および
(75) 発明者/出願人 (米国についてのみ): 塚原克平 (TSUKAHARA, Kappei) [JP/JP]; 〒305-0051 茨城県つくば市二の宮4-4-24 Ibaraki (JP). 畑 桂 (HATA, Katsura) [JP/JP]; 〒305-0035 茨城県つくば市松代1-14-11 サンヒルズ松代405 Ibaraki (JP). 相根康司 (SAGANE, Koji) [JP/JP]; 〒305-0061 茨城県つくば市稲荷前9-7 つくばね第2寮303 Ibaraki (JP). 中本和孝 (NAKAMOTO, Kazutaka) [JP/JP]; 〒305-0061 茨城県つくば市稲荷前9-7 つくばね第2寮304 Ibaraki (JP). 土谷満美子 (TSUCHIYA, Mamiko) [JP/JP]; 〒300-1216 茨城県牛久市神谷6丁目22-1 シエルヒーブB-103 Ibaraki (JP). 渡邉直彰 (WATANABE, Naoaki) [JP/JP]; 〒305-0053 茨城県つくば市小野川7-27 Ibaraki (JP). 大場史記 (OBA, Fuminori) [JP/JP]; 〒177-0045 東京都練馬区石神井台3-1-6-101 Tokyo (JP). 塚田格 (TSUKADA, Itaru) [JP/JP]; 〒300-1222 茨城県牛久市南3-11-13 Ibaraki (JP). 上田教博 (UEDA, Norihiro) [JP/JP]; 〒305-0861 茨城県つくば市谷田部1077-140 Ibaraki (JP). 田中圭悟 (TANAKA, Keigo) [JP/JP]; 〒305-0035 茨城県つくば市松代1-30-12 サンビレッジ松代F202 Ibaraki (JP). 甲斐純子 (KAI, Junko) [JP/JP]; 〒300-4118 茨城県新治郡新治村田土部2084-2 Ibaraki (JP).

[続葉有]

(54) Title: FUNGAL CELL WALL SYNTHESIS GENE

(54) 発明の名称: 真菌の細胞壁合成遺伝子

(57) Abstract: A reporter system reflecting the transport process of GPI anchor protein to cell wall is constructed and a compound inhibiting this process is found out. Further, a gene imparting tolerance to the above compound is identified and a method of screening a compound inhibiting the activity of the protein encoded by this gene is developed. Thus, it is clarified by the novel compound that antifungal agents depending on a novel mechanism, wherein the transport process of GPI anchor protein to cell wall is inhibited, are available.

(57) 要約:

本発明者らは、GPIアンカー蛋白質の細胞壁への輸送過程を反映したレポータ系を作製し、その過程を阻害する化合物を見出した。更に該化合物に対し耐性を付与する遺伝子を同定し、該遺伝子がコードする蛋白質の活性を阻害する化合物のスクリーニング法を開発した。

本発明は、GPIアンカー蛋白質の細胞壁への輸送過程を阻害するという、新規メカニズムの抗真菌剤が可能であることを、新規化合物をもって示した。

WO 02/04626 A1

(74) 代理人: 清水初志, 外(SHIMIZU, Hatsushi et al.); 〒300-0847 茨城県土浦市卸町1-1-1 関鉄つくばビル6階 Ibaraki (JP).

(81) 指定国 (国内): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) 指定国 (広域): ARIPO 特許 (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), ユーラシア特許 (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), ヨーロッパ特許 (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI 特許 (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

添付公開書類:
— 国際調査報告書

２文字コード及び他の略語については、定期発行される各*PCT*ガゼットの巻頭に掲載されている「コードと略語のガイダンスノート」を参照。

明細書

真菌の細胞壁合成遺伝子

技術分野

本発明は、真菌の細胞壁合成に関与する蛋白質をコードするDNA、該DNAがコードする蛋白質、ある化合物がGPIアンカー蛋白質の細胞壁への輸送過程に影響を及ぼすか否かを検定する方法、GPIアンカー蛋白質の細胞壁への輸送過程に影響を及ぼす抗真菌剤に関する。

発明の背景

近年、高度な化学療法等により免疫機能の低下した患者や高齢者の増加により、日和見感染に対する対策は益々重要性を増してきている。カンジダ、アスペルギルス、クリプトコッカス等による内臓真菌感染症はこうした日和見感染症の一部を占め、その割合は年々増加している。異なる弱毒菌による日和見感染が次々と起こっている事実は、患者の抵抗力が低下するような基礎疾患がある限り感染症の問題は後を絶たないことを示している。近い将来確実に訪れる高齢化社会においては、耐性菌の問題を含めた新たな感染症対策が重要な課題の一つとなるにもかかわらず、現状では有効な治療薬がきわめて少ない。

これまでの真菌感染症治療剤は既知の骨格に化学修飾し新規化合物を開発するストラテジーが中心であったが、耐性菌の問題もあり新規メカニズムに基づく新薬の開発が切望されている。

このような現状を踏まえ、発明者らは、未だ充分な治療薬が揃っていない抗真菌剤領域において「病原体が病原性を発揮できないようにすることにより、感染症の発症・進展・持続に対して効果を示す」という新

たなアプローチを試みた。感染を成立・進展させないためには、感染成立の第一段階である宿主への付着、およびその後のコロニゼーションの進展を抑えることが最も効果的であると考えた。そして、「付着因子自体の発現を阻害する」というこれまで行われていない新たなアプローチを実施することにした。

付着因子の発現を阻害するために、発明者らは、「付着因子等の細胞壁表層糖蛋白質は、一度細胞膜にGPI (Glycosylphosphatidylinositol) アンカリングした後、細胞壁表層に輸送される（図１）。」という仮説に着目した。現在までに付着リガンドを含む30種類以上の細胞壁表層糖蛋白質が、GPIアンカリングを介して輸送される（GPIアンカー蛋白質と称す）ことが明らかになっており、この輸送の段階を阻害すれば、付着因子および主要細胞壁構成蛋白の細胞壁表層での発現が阻害される可能性が高いと考えられた。(Hamada K et al, Mol. Gen. Genet., 258: 53-59, 1998)。また、病原性真菌であるカンジダにおいてもGPIアンカー蛋白質の存在が報告されていた（Kapteyn JC et al, Eur. J. Cell Biol., 65:402-407, 1994）。

発明者らは、真菌において細胞膜に存在するGPIアンカー蛋白質が、細胞壁に輸送される過程を阻害することにより、細胞壁合成の阻害による新規抗真菌剤が創出できると考えて、研究に着手した。

発明の開示

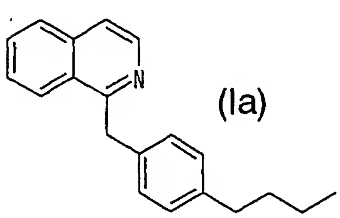
本発明の課題は、細胞壁表層糖蛋白質の発現を阻害し、細胞壁assemblyを阻害するとともに細胞への付着を阻害して、病原体が病原性を発揮できないようにすることにより、感染症の発症・進展・持続に対して効果を示す、抗真菌剤を開発することにある。

GPIアンカー蛋白質の細胞壁への輸送過程を阻害する化合物をスクリ

ーニングするため、本発明者らは、GPIアンカー蛋白質の一つCWP2 (Van Der Vaat JM et al, J. Bacteriol., 177:3104-3110,1995) のC末端にある輸送シグナルとレポータ酵素の融合蛋白質によるレポータ系の作製を試みた。

分泌シグナル遺伝子＋レポータ酵素遺伝子＋CWP2のC末端遺伝子（有りor無し）から成るDNAを構築し、融合蛋白質をSaccharomyces cerevisiae（以下S. cerevisiae）に発現させたところ、レポータ酵素の活性が、CWP2のC末端が有る場合は細胞壁に、無い場合は培養上清中に見出されることが明らかとなった。この結果より、もし被検試料によってGPIアンカー蛋白質の細胞壁への輸送過程が阻害されれば、細胞壁のレポータ酵素の活性が減少する、あるいはレポータ酵素の活性が培養上清中に見出されることが予想され、本レポータ系によるGPIアンカー蛋白質の細胞壁への輸送過程を阻害する化合物のスクリーニングを開始した。

本レポータ系によるスクリーニングより、幾つかのGPIアンカー蛋白質の細胞壁への輸送過程を阻害する化合物が見出された。その代表的な例が、式（Ｉａ）で表される化合物である。



(Ia)

前記式（Ｉａ）で表される化合物は、S. cerevieiae及びCandida albicans（以下C. albicans）の増殖を抑制し、前記式（Ｉａ）で表される化合物存在下で培養したC. albicansは、細胞への付着能が弱く、前記式（Ｉａ）で表される化合物は、GPIアンカー蛋白質の細胞壁への輸送過程を阻害することにより、付着因子の発現を抑制して真菌の付着を阻害するという、当初目的としていた化合物であることが確認された。更に

透過型電子顕微鏡による観察により、前記式（Ｉａ）で表される化合物存在下で培養したC. albicansは、細胞壁の合成に異常があることも確認された。

前記式（Ｉａ）に記載の化合物により、本発明者らは「GPIアンカー蛋白質の細胞壁への輸送過程を阻害する」というメカニズムによる抗真菌剤が可能であることを証明した。

本発明者らは、更に前記式（Ｉａ）で表される化合物が作用している標的蛋白質を特定するため、前記式（Ｉａ）で表される化合物に対し耐性を付与する遺伝子の探索を行った。

S. cerevisiaeに、S. cerevisiae遺伝子のプラスミドライブラリーを導入し、過剰発現により、前記式（Ｉａ）で表される化合物に対して耐性を示すようになったS. cerevisiaeよりプラスミドを回収して、耐性遺伝子をクローニングし、塩基配列を決定して、同遺伝子をGWT1と命名した（配列番号１）。GWT1遺伝子産物を過剰発現させたS. cerevisiaeでは、前記式（Ｉａ）で表される化合物存在下でも、前述のGPIアンカー蛋白質のＣ末端を有するレポータ酵素は、細胞壁へ輸送された。また、前記式（Ｉａ）で表される化合物存在下でも、細胞壁が正常であることが透過型電子顕微鏡観察において確認された。

更に、S. cerevisiaeのゲノムDNA上にランダムに点突然変異を導入し、前記式（Ｉａ）で表される化合物特異的に耐性を示すようになった変異株R1，R5を単離したところ、R1変異株ではGWT1遺伝子の405番目のコドンがGTCからATCに、またR5変異株では140番目のコドンがGGGからAGGに変化する点突然変異が見出された。これら変異GWT1遺伝子をGWT1遺伝子破壊株に導入すると前記式（Ｉａ）で表される化合物に対して耐性を示すことから、この化合物に対する耐性はGWT1遺伝子のみで説明可能なことが明らかとなった。これらのことから、前記式（Ｉａ）で表される化合

物は、GWT1遺伝子産物に直接作用して、GWT1タンパク質の機能を阻害していることが示唆された。

同様な方法により、C. albicansの耐性遺伝子（配列番号３、及び５）もクローニングし塩基配列を決定し、同遺伝子をCaGWT1と命名した。

また、データベースからのGWT1とのホモロジー検索により、Schizosaccharomyces pombe（以下S.pombe）のホモログ（配列番号２７）が見出された。更に、S.cerevisiae, S.pombe, C.albicansのGWT1遺伝子のコードする蛋白において、高度に保存されている領域の配列を基にプライマーを設定してPCRを行うことによりAspergillus fumigatus（以下A.fumigatus）ホモログ（配列番号３９、４１）が見出された。また、データベースからのGWT1とのホモロジー検索により見出された配列を基にPCRを行って、Cryptococcus neoformans（以下C.neoformans）ホモログ（配列番号５４、５８）が見出された。

すなわち本発明は、

１．真菌における過剰発現により、真菌に対し下記式（Ｉａ）で示される化合物に対する耐性を付与する作用を有する蛋白質をコードする、下記（ａ）から（ｅ）のいずれかに記載のDNA。

（ａ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列からなる蛋白質をコードするDNA。

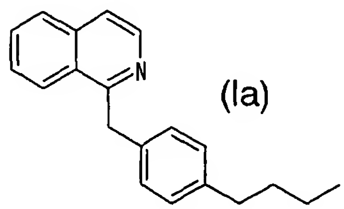
（ｂ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列を含むDNA。

（ｃ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列からなるDNAとストリンジェントな条件下でハイブリダイズするDNA。

（ｄ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列において1若しくは複数のアミノ酸が付加、欠失、置換および／また

は挿入されたアミノ酸配列からなる蛋白質をコードするDNA。

（ｅ）配列番号：２９及び３１あるいは配列番号：２９及び３０をプライマーとして増幅されるDNA。



(Ia)

２．その機能の欠損により真菌の細胞壁におけるGPIアンカー蛋白質量を減少させる作用を有する蛋白質をコードする、下記（ａ）から（ｅ）のいずれかに記載のDNA。

（ａ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列からなる蛋白質をコードするDNA。

（ｂ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列を含むDNA。

（ｃ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列からなるDNAとストリンジェントな条件下でハイブリダイズするDNA。

（ｄ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列において1若しくは複数のアミノ酸が付加、欠失、置換および／または挿入されたアミノ酸配列からなる蛋白質をコードするDNA。

（ｅ）配列番号：２９及び３１あるいは配列番号：２９及び３０をプライマーとして増幅されるDNA。

ここでストリンジェントな条件とは、例えば65℃ ４ x SSCにおけるハイブリダイゼーション、次いで65℃で１時間0.1 x SSC中での洗浄であ

る。また別法としてストリンジェントな条件は、50%ホルムアミド中42℃ 4 x SSCである。また、PerfectHyb™ (TOYOBO) 溶液中65℃2.5時間ハイブリダイゼーション、次いで1).2xSSC, 0.05% SDS溶液：25℃5分、2).2xSSC, 0.05% SDS溶液：25℃15分、3).0.1xSSC, 0.1% SDS溶液50℃20分の洗浄といった条件も許される。

また該DNAを欠失するとは、機能を持った該DNAの遺伝子産物の発現が無い、あるいは発現が減少することを意味し、例えば相同組換えの技術を使って、該DNAのコード領域に無関係なDNA、例えば選択マーカー等を挿入することにより、該DNAを欠失させることを意味する。

真菌細胞壁でのGPIアンカー蛋白質由来の蛋白質は、1).GPIアンカー蛋白質の細胞壁への輸送過程を反映したレポータ系、2).細胞壁中のGPIアンカー蛋白質の一種類を定量するELISA、3).動物細胞への付着といったGPIアンカー蛋白質の活性、4).透過型電子顕微鏡による菌体最外層の綿状線維構造の観察、により定量が可能であり、これらの方法を単独であるいは組合わせて用いることにより、該蛋白質が減少することが確認できる。

3．1または2に記載のDNAによりコードされる蛋白質。

4．1または2に記載のDNAが挿入されたベクター。

5．1または2に記載のDNAまたは4に記載のベクターを保持する形質転換体。

6．3に記載の蛋白質が過剰発現している真菌である、5に記載の形質転換体。

7．3に記載の蛋白質の機能が欠損している真菌

8．5に記載の形質転換体を培養し、該形質転換体またはその培養上清から発現させた蛋白質を回収する工程を含む、3に記載の蛋白質の製造方法。

９．３に記載の蛋白質に結合する抗体。

１０．抗真菌作用を有する化合物をスクリーニングする方法であって、

（ａ）３に記載の蛋白質に被検試料を接触させる工程、

（ｂ）該蛋白質と被検試料との結合活性を検出する工程、

（ｃ）該蛋白質に結合する活性を有する化合物を選択する工程、を含む方法。

１１．抗真菌作用を有する化合物をスクリーニングする方法であって、

（ａ）３に記載の蛋白質が過剰発現している真菌に被検試料を接触させる工程、

（ｂ)該真菌におけるGPIアンカー蛋白質の細胞壁への輸送量を検出する工程、

（ｃ）３に記載の蛋白質が過剰発現していない真菌に被検資料を接触させた場合と比較して、工程（ｂ）において検出されるGPIアンカー蛋白質の細胞壁への輸送量を減少させる化合物を選択する工程、を含む方法。

ここで被検試料によるGPIアンカー蛋白質の細胞壁への輸送量の減少は、例えば増殖速度の低下、膨化、温度感受性、細胞壁でのGPIアンカー蛋白質由来の蛋白質の減少等により検出することが可能であるが、好ましくは、細胞壁でのGPIアンカー蛋白質由来の蛋白質の減少により検出することが望ましい。

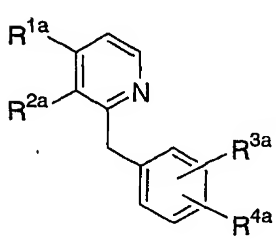
GPIアンカー蛋白質由来の蛋白質の減少は、1).GPIアンカー蛋白質の細胞壁への輸送過程を反映したレポータ系、2).細胞壁中のGPIアンカー蛋白質の一種類を定量するELISA、3).動物細胞への付着といったGPIアンカー蛋白質の活性、4).透過型電子顕微鏡による菌体最外層の綿状線維構造の観察、により定量が可能であり、これらの方法を単独であるいは組合わせて用いることにより、GPIアンカー蛋白質の細胞壁への輸送量の減少が検定できる。

１２．前記１０または１１に記載のスクリーニングにより単離しうる、抗真菌作用を有する化合物。

１３． 真菌においてGPIアンカー蛋白質の細胞壁への輸送を阻害する化合物を有効成分とする抗真菌剤。

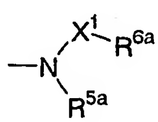
１４．９に記載の抗体または前記１２に記載の化合物を有効成分とする、抗真菌剤。

１５．一般式(I)



(I)

[式中R^{1a}およびR^{2a}は同一または相異なってそれぞれ水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、トリフルオロメチル基、トリフルオロメトキシ基、置換されてもよいC_{1-6}アルキル基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、置換されてもよいC_{1-6}アルコキシ基、または式

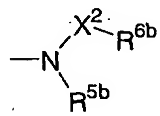


（式中X^1は単結合、カルボニル基、または式 $-S(O)_2-$ で表わされる基を意味する；

R^{5a}およびR^{6a}は同一または相異なって、水素原子、または置換されていてもよいC_{1-6}アルキル基を意味する）で表わされる基を示す。また、R^{1a}とR^{2a}は一緒になって、置換されていてもよいベンゼン環、置換されていてもよいピリジン環、置換されていてもよいピロール環、置換されていてもよいチオフェン環、置換されていてもよいフラン環、置換されていてもよいピリダジン環、置換されていてもよいピリミジン環、置換されていてもよいピラジン環、置換されていてもよいイミダゾール環、置換

されていてもよいオキサゾール環、置換されていてもよいチアゾール環、置換されていてもよいピラゾール環、置換されていてもよいイソオキサゾール環、置換されていてもよいイソチアゾール環、置換されていてもよいシクロヘキサン環、および置換されていてもよいシクロペンタン環からなる群から選ばれる縮合環を形成してもよい；

R^{3a}、およびR^{4a}は同一または相異なってそれぞれ、水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、カルボキシシル基、ホルミル基、ヒドロキシイミノ基、トリフルオロメチル基、トリフルオロメトキシ基、C_{1-6}アルキル基、C_{1-6}アルコキシ基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、式 $-C(O)NR^{7a}R^{7b}$（式中、R^{7a}およびR^{7b}は同一または相異なってそれぞれ水素原子、またはC_{1-6}アルキル基を意味する）、式 $-CO_2R^{7a}$（式中、R^{7a}は前記定義と同意義を意味する）、式 $-S(O)_nR^{7a}$（式中、nは０ないし２の整数を意味する。R^{7a}は前記定義と同意義を意味する）、式 $-S(O)_2NR^{7a}R^{7b}$（式中、R^{7a}およびR^{7b}は前記定義と同意義を意味する）、式



（式中X^2は単結合、カルボニル基、または式 $-S(O)_2-$ で表わされる基を意味する；

R^{5b}およびR^{6b}は同一または相異なっていて、水素原子、置換されていてもよいC_{1-6}アルキル基、または置換されていてもよいC_{6-14}アリール基を意味する）で表わされる基、または式

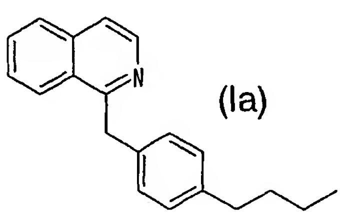
$-Z^1-Z^2$

（式中、Z^1は単結合、酸素原子、ビニレン基、またはエチニレン基を意味する；

Z^2は単結合、または０ないし４個の置換基で置換されてもよいC_{1-6}アルキル基を意味する）で表わされる基を意味する。R^{3a}とR^{4a}は一緒になって、

メチレンジオキシ基、または1,2-エチレンジオキシ基を意味してもよく、またR^{3a}とR^{4a}は一緒になって、置換されていてもよいベンゼン環、置換されていてもよいピリジン環、置換されていてもよいピロール環、置換されていてもよいチオフェン環、置換されていてもよいフラン環、置換されていてもよいピリダジン環、置換されていてもよいピリミジン環、置換されていてもよいピラジン環、置換されていてもよいイミダゾール環、置換されていてもよいオキサゾール環、置換されていてもよいチアゾール環、置換されていてもよいピラゾール環、置換されていてもよいイソオキサゾール環、置換されていてもよいイソチアゾール環、置換されていてもよいシクロヘキサン環および置換されていてもよいシクロペンタン環からなる群から選ばれる縮合環の形成を意味してもよい。ただし、R^{1a}およびR^{2a}がともに水素原子を意味する場合は除く。〕で示される化合物もしくはその塩またはそれらの水和物を有効成分とする前記１３．に記載の抗真菌剤。

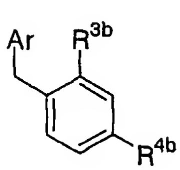
１６．式



(Ia)

で表される化合物（Ｉａ）を有効成分とする前記１３．に記載の抗真菌剤。

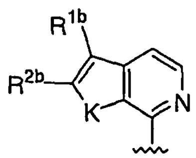
１７．一般式(II)



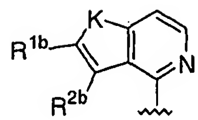
(II)

〔式中Arは下記式（IIIa）－（IIIf）からなる群

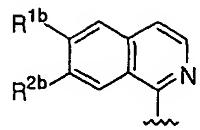
(IIIa)



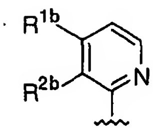
(IIIb)



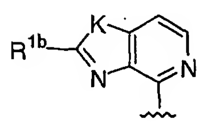
(IIIc)



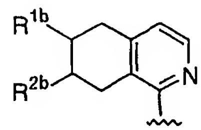
(IIId)



(IIIe)

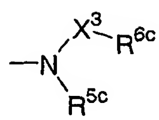


(IIIf)



（式中、Kは硫黄原子、酸素原子、または式 -NH- で表わされる基を意味する；

R^{1b}、R^{2b}は同一または相異なってそれぞれ水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、トリフルオロメチル基、トリフルオロメトキシ基、式



（式中X^3は単結合、カルボニル基、または式 $-S(O)_2-$ で表わされる基を意味する；

R^{5c}およびR^{6c}は同一または相異なっていて、水素原子、置換されていてもよいC_{1-6}アルキル基を意味する）で表わされる基、または式 $-X^4-R^{8a}$（式中、 X^4は、単結合、酸素原子、または硫黄原子を意味する；R^{8a}はC_{1-6}アルキル基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、C_{3-8}シクロアルキル基、またはC_{3-8}シクロアルケニル基を意味する）で表わされる基を示す。また、R^{1b}、R^{2b}は一緒になってメチレンジオキシ基、または1,2-エチレンジオキシ基を形成してもよい。）から選ばれる置換基を意味する；

R^{3b}、およびR^{4b}は同一または相異なってそれぞれ、水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、カルボキシル基、ホルミル基、ヒ

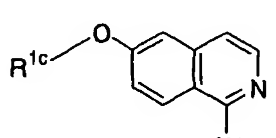
ドロキシイミノ基、トリフルオロメチル基、トリフルオロメトキシ基、C_{1-6}アルキル基、C_{1-6}アルコキシ基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、または式、

$-Z^{1b}-Z^{2b}$

（式中、Z^{1b}は単結合、ビニレン基、またはエチニレン基を意味する；Z^{2b}は単結合、または０ないし４個の置換基で置換されてもよいC_{1-6}アルキル基を意味する）で表わされる基を意味する。；

ただし（１） Arが、R^{1b}およびR^{2b}がともに水素原子である前記式（IIId）で表わされる場合、（２） R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水素原子、メトキシ基、水酸基、メチル基、ベンジルオキシ基、またはハロゲン原子であり、 Arが、R^{1b}およびR^{2b}がともに水素原子またはメトキシ基を意味する前記式（IIIc）で表わされる場合、（３） R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水素原子、水酸基、メトキシ基、またはベンジルオキシ基であり、 Arが、R^{1b}およびR^{2b}がともに水酸基またはベンジルオキシ基を意味する前記式(IIIc)で表わされる場合、または（４）Arが、R^{1b}が水素原子でR^{2b}がホルミル基、ヒドロキシメチル基またはメトキシカルボニル基である前記式(IIId) で表わされる場合を除く。〕で示される化合物もしくはその塩またはそれらの水和物

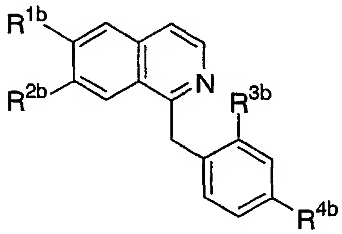
１８．Arが式、



(IIIg)

（式中、R^{1c}が水素原子、置換されてもよいC_{1-6}アルキル基、ベンジル基を意味する）で表わされ、かつR^{3b}が水素原子を意味する場合を除いた、１７．記載の化合物もしくはその塩またはそれらの水和物

１９． 一般式(IIIc2)

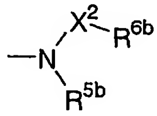


(IIIc2)

〔式中R^{1b}、R^{2b}は前記定義と同意義を意味する。ただし、（１）R^{1b}が式R^{1c}-O-（式中、R^{1c}は前記定義と同意義を意味する）で表わされる基であり、R^{2b}が水素原子であり、R^{3b}が水素原子を意味する場合、（２）R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水素原子、メトキシ基、水酸基、メチル基、ベンジルオキシ基、またはハロゲン原子であり、R^{1b}およびR^{2b}がともに水素原子またはメトキシ基を意味する場合、または（３）R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水素原子、水酸基、メトキシ基、またはベンジルオキシ基であり、R^{1b}およびR^{2b}がともに水酸基またはベンジルオキシ基を意味する場合を除く。〕で表される化合物もしくはその塩またはそれらの水和物

２０．抗真菌作用を有する前記１７．記載の抗真菌剤

２１．R^{3a}、およびR^{4a}のうち少なくとも１つが、式 -C(O)NR^{7a}R^{7b}（式中、R^{7a}およびR^{7b}は前記定義と同意義を意味する）、式 -CO$_2$R^{7a}（式中、R^{7a}は前記定義と同意義を意味する）、式 -S(O)$_n$R^{7a}（式中、nは０ないし２の整数を意味する。R^{7a}は前記定義と同意義を意味する）、式 -S(O)$_2$NR^{7a}R^{7b}（式中、R^{7a}およびR^{7b}は前記定義と同意義を意味する）、式



（式中X^2、R^{5b}およびR^{6b}は前記定義と同意義を意味する）で表わされる基、または０ないし４個の置換基で置換されてもよいC_{1-6}アルコキシ基を意味し、またはR^{3a}とR^{4a}は一緒になって、メチレンジオキシ基、または1,2-

エチレンジオキシ基を意味する前記１５．記載の抗真菌剤

２２．抗真菌作用を有する化合物が、（１）1-ベンジルイソキノリン、（２）1-(4-ブロモベンジル)イソキノリン、（３）1-(4-クロロベンジル)イソキノリン、（４）1-(4-フルオロベンジル)イソキノリン、（５）1-(4-ヨードベンジル)イソキノリン、（６）1-(3-メチルベンジル)イソキノリン、（７）1-(4-メチルベンジル)イソキノリン、（８）1-(3,4-ジメチルベンジル)イソキノリン、（９）1-(3-メトキシベンジル)イソキノリン、（１０）1-(4-メトキシベンジル)イソキノリン、（１１）1-(3,4-メチレンジオキシベンジル)イソキノリン、（１２）1-(4-ベンジルオキシベンジル)イソキノリン、（１３）1-(4-シアノベンジル)イソキノリン、（１４）1-(4-ニトロベンジル)イソキノリン、（１５）1-(4-アミノベンジル)イソキノリン、（１６）1-(4-メトキシベンジル)-6,7-ジクロロ-イソキノリン、（１７）1-(4-メトキシ-2-ニトロ-ベンジル)-イソキノリン、（１８）1-(4-メトキシベンジル)-6,7-メチレンジオキシ-イソキノリン、（１９）1-(2-アミノ-4-メトキシ-ベンジル)イソキノリン、（２０）1-(4-メトキシベンジル)-7-ヒドロキシ-6-メトキシ-イソキノリン、（２１）1-(4-ベンジルオキシベンジル)-6,7-ジメトキシ-イソキノリン、（２２）1-(4-メトキシベンジル)-6,7-ジメトキシ-イソキノリン、（２３）1-(4-メトキシ-2-ニトロ-ベンジル)-イソキノリン、（２４）3-[4-(1-イソキノリルメチル)フェノキシ]プロピルシアニド、（２５）1-[4-(2,2,3,3-テトラフルオロプロポキシ)ベンジル]イソキノリン、（２６）1-[4-(2-ピペリジノエトキシ)ベンジル]イソキノリン、（２７）4-(1-イソキノリルメチル)フェニル(2-モルフォリノエチル)エーテル、（２８）1-[4-(2-メトキシエトキシ)ベンジル]イソキノリン、（２９）*N*-{2-[4-(1-イソキノリルメチル)フェノキシ]エチル}-*N*,*N*-ジメチルアミン、（３０）1-[4-(フェネチルオキシ)ベンジル]イソキノリン、（３１）1-{4-[(2

-メチルアリル)オキシ]ベンジル}イソキノリン、（３２）1-(4-イソブトキシベンジル)イソキノリン、（３３）1-[4-(2-フェノキシエトキシ)ベンジル]イソキノリン、（３４）メチル2-[4-(1-イソキノリルメチル)フェノキシ]アセテート、（３５）2-[4-(1-イソキノリルメチル)フェノキシ]-1-エタノール、（３６）t-ブチル*N*-{2-[4-(1-イソキノリルメチル)フェノキシ]エチル}カーバメート、（３７）1-{4-[3-(テトラヒドロ-2H-2-ピラニルオキシ)プロポキシ]ベンジル}イソキノリン、（３８）2-[4-(1-イソキノリルメチル)フェノキシ] -1-エタンアミン、（３９）1-[4-(3-ピペリジノプロポキシ)ベンジル]イソキノリン、（４９）3-[4-(1-イソキノリルメチル)フェノキシ]-1-プロパノール、（４１）1-[4-(2-エチルブトキシ)ベンジル]イソキノリン、（４２）4-[4-(1-イソキノリルメチル)フェノキシ]ブタノイックアシッド、（４３）1-(4-{3-[(4-ベンジルピペラジノ)スルフォニル]プロポキシ}ベンジル)イソキノリン、（４４）1-(4-{3-[4-(4-クロロフェニル)ピペラジノ]プロポキシ}ベンジル)イソキノリン、（４５）4-(1-イソキノリルメチル)アニリン、（４６）*N*-[4-(1-イソキノリルメチル)フェニル]ブタンアミド、（４７）*N*-[4-(1-イソキノリルメチル)フェニル]プロパンアミド、（４８）*N*-[4-(1-イソキノリルメチル)フェニル]-1-エタンスルフォンアミド、（４９）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-メチル-エタンスルフォンアミド、（５０）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-メチルアミン、（５１）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-プロピルアミン、または（５２）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-メチル-*N*-プロピルアミンである前記１５．記載の抗真菌剤

２３．治効量の請求項１３から２２のいずれかに記載の抗真菌剤を哺乳動物に投与することを含む、真菌感染症の治療方法、に関する。

以下に、本願明細書において記載する用語、記号等の意義を説明し、

本発明を詳細に説明する。

なお、本願明細書中においては、化合物の構造式が便宜上一定の異性体を表すことがあるが、本発明には化合物の構造上生ずる総ての幾何異性体、不斉炭素に基づく光学異性体、立体異性体、互変異性体等の異性体および異性体混合物を含み、便宜上の式の記載に限定されるものではなく、いずれか一方の異性体でも混合物でもよい。従って、分子内に不斉炭素原子を有し光学活性体およびラセミ体が存在することがあり得るが、本発明においては特に限定されず、いずれの場合も含まれる。さらに結晶多形が存在することもあるが同様に限定されず、いずれかの結晶形単一または混合物であってもよく、また、無水物であっても水和物であってもどちらでもよい。

また本発明化合物が、生体内で酸化、還元、加水分解、または抱合などの代謝を受けて抗真菌作用を示す化合物も含有する。またさらに、本発明は生体内で酸化、還元、加水分解などの代謝を受けて本発明化合物を精製する化合物をも含有する。

本明細書中において表される「C_{1-6}アルキル基」とは、炭素数1ないし6個の直鎖状または分枝鎖状のアルキル基を意味し、具体的には例えばメチル基、エチル基、n-プロピル基、i-プロピル基、n-ブチル基、i-ブチル基、tert-ブチル基、n-ペンチル基、i-ペンチル基、ネオペンチル基、n-ヘキシル基、1-メチルプロピル基、1,2-ジメチルプロピル基、2-エチルプロピル基、1-メチル-2-エチルプロピル基、1-エチル-2-メチルプロピル基、1,1,2-トリメチルプロピル基、1-メチルブチル基、2-メチルブチル基、1,1-ジメチルブチル基、2,2-ジメチルブチル基、2-エチルブチル基、1,3-ジメチルブチル基、2-メチルペンチル基、3-メチルペンチル基等があげられる。

本明細書中において表される「C_{2-6}アルケニル基」とは、炭素数2

ないし6個の直鎖状または分枝鎖状のアルケニル基を意味し、具体的には例えばビニル基、アリル基、1-プロペニル基、イソプロペニル基、1-ブテン-1-イル基、1-ブテン-2-イル基、1-ブテン-3-イル基、2-ブテン-1-イル基、2-ブテン-2-イル基等があげられる。

本明細書中において表される「C_{2-6}アルキニル基」とは、炭素数2ないし6個の直鎖状または分枝鎖状のアルキニル基を意味し、具体的には例えば、エチニル基、1-プロピニル基、2-プロピニル基、ブチニル基、ペンチニル基、ヘキシニル基等があげられる。

本明細書中において表される「C_{1-6}アルコキシ基」とは前記定義の「C_{1-6}アルキル基」が結合したオキシ基であることを意味し、具体的には、例えばメトキシ基、エトキシ基、n-プロポキシ基、i-プロポキシ基、n-ブトキシ基、i-ブトキシ基、sec-ブトキシ基、t-ブトキシ基、n-ペンチルオキシ基、i-ペンチルオキシ基、sec-ペンチルオキシ基、t-ペンチルオキシ基、ネオペンチルオキシ基、1-メチルブトキシ基、2-メチルブトキシ基、1,1-ジメチルプロポキシ基、1,2-ジメチルプロポキシ基、n-ヘキシルオキシ基、i-ヘキシルオキシ基、1-メチルペンチルオキシ基、2-メチルペンチルオキシ基、3-メチルペンチルオキシ基、1,1-ジメチルブトキシ基、1,2-ジメチルブトキシ基、2,2-ジメチルブトキシ基、1,3-ジメチルブトキシ基、2,3-ジメチルブトキシ基、3,3-ジメチルブトキシ基、1-エチルブトキシ基、2-エチルブトキシ基、1,1,2-トリメチルプロポキシ基、1,2,2-トリメチルプロポキシ基、1-エチル-1ーメチルプロポキシ基、1-エチル-2ーメチルプロポキシ基などが挙げられる。

本明細書中において表される「C_{6-14}アリール基」とは、炭素数6ないし14の芳香族環基をいい、具体的には例えば、フェニル基、1-ナフチル基、2-ナフチル基、as-インダセニル基、s-インダセニル基、アセナフチレニル基などが挙げられる。

本明細書中において表わされる「ハロゲン原子」とは、フッ素原子、塩素原子、臭素原子、ヨウ素原子を意味する。

本明細書中において表わされる「置換されていてもよい」とは、「置換可能な部位に、任意に組み合わせて1または複数個の置換基を有してもよい」と同意義であり、置換基は具体的には例えば、水素原子、ハロゲン、ニトロ基、シアノ基、ヒドロキシル基、メルカプト基、ヒドロキシアルキル基、カルボキシル基、C_{1-6}アルコキシカルボニル基、C_{2-7}アシルアミノ基、C_{1-6}アルキルアミノ基、ピリジル基、C_{1-6}アルキルスルフィニル基、C_{1-6}アルキルスルフォニル基、C_{1-6}アルキルスルファモイル基、C_{1-6}アルキルスルフィナモイル基、C_{1-6}アルキルスルフェナモイル基、テトラヒドロピラニル基、C_{1-6}アルキルカルバモイル基、または式 $-X^4-R^{8a}$(式中、X^4は、単結合、酸素原子、または硫黄原子を意味する；R^{8a}はC_{1-6}アルキル基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、C_{6-14}アリール基、C_{3-8}シクロアルキル基、またはC_{3-8}シクロアルケニル基を意味する）などが挙げられる。

本明細書中において表わされる「0ないし4個の置換基で置換されていてもよい」とは、「置換可能な部位に、任意に組み合わせて1または4個の置換基を有してもよい」と同意義であり、置換基は前記定義と同意義である。

本発明における「塩」とは薬理学的に許容される塩を示し、本発明化合物と付加塩を形成したものであれば特に限定されないが、好ましい例としては、フッ化水素酸塩、塩酸塩、臭化水素酸塩、ヨウ化水素酸塩などのハロゲン化水素酸塩；硫酸塩、硝酸塩、過塩素酸塩、リン酸塩、炭酸塩、重炭酸塩などの無機酸塩；酢酸塩、シュウ酸塩、マレイン酸塩、酒石酸塩、フマル酸塩などの有機カルボン酸塩；メタンスルホン酸塩、トリフルオロメタンスルホン酸塩、エタンスルホン酸塩、ベンゼンスル

ホン酸塩、トルエンスルホン酸塩、カンファースルホン酸塩などの有機スルホン酸塩；アスパラギン酸塩、グルタミン酸塩などのアミノ酸塩；トリメチルアミン塩、トリエチルアミン塩、プロカイン塩、ピリジン塩、フェネチルベンジルアミン塩などのアミンとの塩；ナトリウム塩、カリウム塩などのアルカリ金属塩；マグネシウム塩、カルシウム塩などのアルカリ土類金属塩等があげられる。

以下に本発明に記載された、1．細胞壁合成に関与する蛋白質をコードするDNAを得る方法、2．被検試料がGPIアンカー蛋白質の細胞壁への輸送過程に影響を及ぼすか否かを検定する方法、3．前記式（Ｉａ）の化合物を得る方法について開示する。

1．真菌の細胞壁合成に関与する蛋白質をコードするDNAを得る方法

以下に、(1).真菌に過剰発現することにより、前記式（Ｉａ）に記載の化合物に対する耐性を獲得する蛋白質をコードするDNAを得る方法、(2).配列番号1、配列番号3あるいは配列番号5に記載のDNAとストリンジェントな条件でハイブリダイズするDNAを得る方法、(3).ホモロジー検索を基に、真菌の細胞壁合成に関与する蛋白質をコードするDNAを得る方法、(4). 前記式（Ｉａ）に記載の化合物に対する耐性を獲得する蛋白質を過剰発現、あるいは欠失した真菌を得る方法について述べる。

(1).真菌に過剰発現することにより、前記式（Ｉａ）に記載の化合物に対する耐性を獲得する蛋白質をコードするDNAを得る方法

ここで真菌とは、接合菌・子嚢菌・担子菌・不完全菌門に属すもので、好ましくは病原性真菌、Mucor・Saccharomyces・Candida・Cryptococcus・Trichosporon・Malassezia・Aspergillus・Trichophyton・Microsporum・Sporothrix・Blastmyces・Coccidioides・Paracoccidioides・Penicillinium・Fusariumであり、更に好ましくはC. albicans・C. glabrata、C. neoformans及びA. fumigatusである。遺伝的な解析の容易なS.

cerevisiae及びS. pombeも好ましい菌種である。

真菌に、当該真菌遺伝子のプラスミドライブラリーを導入する。S. cerevisiae及びS. pombeのプラスミドライブラリーはATCC(Information for ATCC Number: 37323)から入手可能であり、C. albicansのプラスミドライブラリーはNavaro-Garcia F et al, Mol. Cell. Biol., 15: 2197-2206, 1995に記載の方法により作製可能である。得られたプラスミドライブラリーは、Gietz D et al, Nucl. Acids Res. 20: 1425, 1992に記載の方法により真菌に導入する。あるいは、YEASTMAKER™ Yeast Transformation System(Clontech)等のキットを使うことも許される。

プラスミドライブラリーを導入した真菌は、前記式（Ｉａ）に記載の化合物の存在下で培養する。具体的には、前記式（Ｉａ）に記載の化合物を1.56μg/mlから25μg/ml、好ましくは1.56μg/mlから6.25μg/ml、更に好ましくは3.125μg/mlの濃度に含む寒天培地上にプラスミドライブラリーを導入した真菌を接種し、適当な時間、30℃から42℃で２日から５日、好ましくは37℃で３日間培養する。増殖してきたコロニーを、前記式（Ｉａ）に記載の化合物を含む培地中で更に培養し、増殖させた菌体よりプラスミドを精製する。プラスミドの精製は、例えばMETHODS IN ENZYMOLOGY, Vol. 194: 169-182 (1991)に記載の方法に行うことができる。

得られたプラスミドは、好ましくは直接塩基配列を決定するが、必要で有れば、適当なベクター、例えば塩基配列の決定に適したpBluescript II、pUC19等にリクローニングを行い、塩基配列を決定する。塩基配列の決定は、例えばABI377 system (PE apllied Biosystems社製) マニュアルに記載の方法で行うことができる。

本発明の実施例においては、S. cerevisiaeでは独立に取得した27コロニーの全てが、C. albicansでは30コロニー中28コロニーが、本発明

に記載のDNAを含んでいた。前記式（Ｉａ）に記載の化合物に対して耐性を付与する遺伝子は、該真菌にただ一つ存在し、上記の方法により取得することが可能である。

(2).配列番号１、配列番号３あるいは配列番号５に記載のDNAとストリンジェントな条件でハイブリダイズするDNAを得る方法

本発明に記載の、真菌の細胞壁合成に関与する蛋白質をコードするDNAを得る方法としては、例えばS. cerevisiaeの遺伝子DNAを鋳型とし、配列番号１に記載の塩基配列の情報よりプライマーを設計して、あるいはC. albicansの遺伝子DNAを鋳型とし、配列番号３あるいは配列番号５に記載の塩基配列の情報よりプライマーを設計して、PCRを行い、増幅されたDNAを適当なベクター、例えばpBlueScript等にクローニングすることにより得る方法が挙げられる。プライマーは増幅したい領域に応じて適宜設計するが、好ましくは15 bp以上、更に好ましくは20 bp以上の長さが望ましく、場合によっては制限酵素部位等、後のDNA構築に必要な配列を付加しても構わない。PCRの条件はプライマーの長さ、増幅する領域の長さ、用いる鋳型DNAの量等に合わせ適宜決定できる。例えばC. albicansの遺伝子DNA 200 ngを鋳型とし、配列番号２１及び配列番号２２をプライマーとして94℃4分→（94℃30秒→68℃5分）x 35サイクル→72℃4分の条件で、真菌の細胞壁合成に関与する蛋白質をコードするDNAを得ることができる。

PCRで得られたDNAは、細胞壁合成に関与する蛋白質をコードするDNAとホモロジーのある、他類の真菌のDNAを得るためのプローブとしても使用することができる。具体的には、例えばS. cerevisiaeの細胞壁合成に関与する蛋白質をコードする、C. albicansの相同遺伝子を得るために、C. albicansの遺伝子ライブラリーあるいはc-DNAライブラリーから、S. cerevisiaeの遺伝子DNAを鋳型としてPCRで得られたDNAをプローブ

とし、ストリンジェントな条件で、ハイブリダイズするDNAをクローニングを行うことができる。ここでストリンジェントな条件とは、例えば65℃ 4 x SSCにおけるハイブリダイゼーション、次いで65℃で1時間0.1 x SSC中での洗浄である。また別法としてストリンジェントな条件は、50%ホルムアミド中42℃ 4 x SSCである。また、PerfectHyb™ (TOYOBO) 溶液中65℃2.5時間ハイブリダイゼーション、次いで1).2xSSC, 0.05% SDS溶液：25℃5分、2).2xSSC, 0.05% SDS溶液：25℃15分、3).0.1xSSC, 0.1% SDS溶液50℃20分の洗浄といった条件も許される。

本発明の実施例では、サザンブロット解析により、C. albicansには配列番号1に記載するDNAとハイブリダイズする遺伝子が1つだけ存在することが明らかとなっており、更に該遺伝子をクローニングしたことが示されている。上記方法により、配列番号1あるいは配列番号3とハイブリダイズするDNAを取得することが可能である。

(3).ホモロジー検索を基に、真菌の細胞壁合成に関与する蛋白質をコードするDNAを得る方法

本発明により、S. cerevisiae, C. albicans, S. pombe, A. fumigatus及びC. neoformansのGWT1ホモログが明らかとなっている。これら遺伝子の間で保存されている領域は、GWT1遺伝子産物が機能を発揮するために重要であると考えられ、これ以外の真菌においても保存されている可能性が高い。

そこで、保存されている領域のアミノ酸配列を基に、プローブを作製してハイブリダイズを行う、あるいはプライマーを設定してPCRを行うことにより、真菌の細胞壁合成に関与する蛋白質をコードするDNAを得ることができる。PCRのプライマーは、保存されている領域をコードするように設定されれば、如何なる配列も許されるが、好ましくは配列番号29及び31あるいは配列番号29及び30が望ましい。

また別法としては、データベースに登録された遺伝子断片から、GWT1とホモロジーを示す塩基配列を探し出し、その塩基配列を基にプライマーを設定して、cDNAより、あるいはゲノムDNAよりPCRを行うことにより、真菌の細胞壁合成に関与する蛋白質をコードするDNAを得ることができる。

得られた配列を基に、全長遺伝子を得るPCRの方法としては、3' RACE・5' RACE・inverse PCR等の手法が挙げられ、またハイブリダイズにより隣接した配列を含むクローンを選択することも可能である。これらの手法を組合わせることにより、全長遺伝子を得ることができる。

(4). 前記式（Ｉａ）に記載の化合物に対する耐性を獲得する蛋白質を過剰発現、あるいは欠失した真菌を得る方法

本発明に記載の、前記式（Ｉａ）に記載の化合物に対する耐性を獲得する蛋白質を過剰発現した真菌、好ましくはS. cerevisiaeは、該蛋白質を発現する発現ベクター、例えば真菌で強制発現が可能なプロモーター、好ましくは出芽酵母エノラーゼ遺伝子（ENO1）のプロモーターの下流に、配列番号１に記載のDNAをつないだ発現ベクターを、真菌染色体上のある特定の位置に挿入する方法により得られる。

挿入する方法は、例えばpRS304 (Sikorski RS et al, Genetics. 122(1): 19-27, 1989) のマルチクローニングサイトに挿入したい配列を挿入し、インテグレーション用ベクターを作製して、真菌に導入することにより行うことができる。詳しい方法はMETHODS IN ENZYMOLOGY Vol. 194: 281-301 (1991)を参照できる。

またC. albicansの過剰発現株は、C. albicans用発現ベクター、例えばpCARS1、pRM1等 (Pla J et al, Yeast 12: 1677-1702, 1996) に配列番号３あるいは配列番号５に記載の遺伝子を組み込んでC. albicansに形質転換する(Sanglard D et al, Antimicrobiol. Agents Chemother.

40: 2300-2305, 1996）ことにより得られる。

本発明に記載の、前記式（Ｉａ）に記載の化合物に対する耐性を獲得する遺伝子を欠失した真菌、好ましくはS. cerevisiaeは、以下の方法により得ることができるが、この例示によって本発明は限定されない。

マーカー遺伝子、好ましくはS. pombeのhis5遺伝子を鋳型とし、両端に30 bp以上好ましくは40 bp以上の欠失したい遺伝子、S. cerevisiaeの場合配列番号１に記載の遺伝子の配列を含んだPCR産物が得られるように設計したプライマーを用いPCR増幅を行う。PCR産物を精製し、真菌に導入後、マーカー遺伝子に対応した選択、his5であればhis⁻の培地で培養して、欠失株を得ることができる。

また、C. albicansの欠失株は、配列番号３あるいは配列番号５に記載の塩基配列情報を基に、hisG-URA3-hisGカセットを用いた常法（Fonzi WA et al, Genetics 134: 717-728,1993）により得られる。

２.被検試料がGPIアンカー蛋白質の細胞壁への輸送過程に影響を及ぼすか否かを検定する方法

被検試料が、GPIアンカー蛋白質の細胞壁への輸送過程を阻害するか否か、あるいはGPIアンカー蛋白質の真菌表層への発現を阻害するか否かは、(1).レポータ酵素を用いる方法、(2).真菌細胞壁の表層糖蛋白質と反応する抗体を用いる方法、(3).動物細胞に対する付着能により検定する方法、(4).真菌を光学顕微鏡あるいは電子顕微鏡で観察する方法により検定できる。

以下に説明する(1)～(4)の方法により、好ましくは(1)～(4)の方法を組み合わせて用いることにより、被検試料がGPIアンカー蛋白質の細胞壁への輸送過程を阻害する、あるいはGPIアンカー蛋白質の真菌表層への発現を阻害すると判断され、しかも本件発明に記載のDNAがコードする蛋白質を、真菌に過剰発現させることにより、その阻害の程度が減弱する、

あるいは阻害が見られなくなる場合に、被検試料は、GPIアンカー蛋白質の細胞壁への輸送過程に影響を与えたと判断される。

以下、(1)～(4)の方法を説明する。

(1).レポータ酵素を用いる方法

GPIアンカー蛋白質の細胞壁への輸送過程は、例えばGPIアンカー蛋白質を放射性同位元素で標識し、真菌細胞壁画分を分画後、GPIアンカー蛋白質に対する抗体による免疫沈降を行うといったトレーサー実験により定量することが可能である。また、より容易には、GPIアンカー蛋白質に共通して見られ、輸送のシグナルとして働いていると考えられるC末端配列を、測定の容易な酵素との融合蛋白質（レポータ酵素）として発現させ、真菌細胞壁画分を分画後、各画分の酵素活性を測定するレポータ系により定量することが可能である（Van Berkel MAA et al, FEBS Letters, 349: 135-138, 1994）。以下にレポータ酵素を用いた方法について説明するが、これは本発明を限定するものではない。

先ず、レポータ遺伝子を構築し真菌に導入する。レポータ遺伝子は、真菌で働くプロモータ配列に続き、それぞれシグナル配列・レポータ酵素・GPIアンカー蛋白質C末端配列をコードするDNAを、reading frameを合わせてつなぎ合わせて構築する。プロモータ配列としては、例えばGAL10、ENO1のプロモータの配列等が挙げられる。シグナル配列としては、例えばα-ファクター、インベルターゼ、リゾチームのシグナル配列等が挙げられる。レポータ酵素としては、例えばβラクタマーゼ・リゾチーム・アルカリホスファターゼ・βガラクトシダーゼ等が挙げられる。酵素活性は持たないが容易に検出が可能なGreen Fluorescence Protein (GFP)を用いても良い。GPIアンカー蛋白質C末端配列としては、例えばα-agglutininC末端配列・CWP2C末端配列等が挙げられる。また、構築したレポータ遺伝子を含むベクター中に、適当な選択マーカ、例えばLE

U2、URA3等を挿入しておくことが好ましい。

構築したレポータ遺伝子を適当な方法、例えば酢酸リチウム法（Gietz D et al, Nucl. Acids Res. 20: 1425, 1992）により真菌に導入し、必要であれば選択マーカに適した方法、LEU2であればLeu⁻の培地、URA3であればUra⁻の培地で培養し、DNAが導入された真菌を選択する。

被検試料がGPIアンカー蛋白質の細胞壁への輸送過程に影響を与えるか否かは、以下の方法により検定する。

レポータ遺伝子を導入した真菌を、被検試料の存在下、適当な条件、例えば30℃で48時間培養する。培養後、培養上清を遠心分離し、培養上清画分のレポータ酵素の活性を測定する。残された菌体画分は、洗浄後、適当な方法例えばグルカナーゼで細胞壁グルカンを分解することにより、細胞壁成分を分離し、細胞壁画分及び細胞質画分のレポータ酵素の活性を測定する。なおアッセイを簡便に行うため、遠心分離後、菌体の洗浄は行わずに、菌体画分中に残る培養上清画分由来のレポータ酵素量を比例計算により求め、菌体画分のレポータ酵素量から差し引いて菌体画分中のレポータ酵素量とすることも許される。

被検試料に、一細胞当たりの培養上清画分中のレポータ酵素活性を上昇させる、あるいは一細胞当たりの細胞壁画分中のレポータ酵素活性を低下させる活性が認められれば、該被検試料はGPIアンカー蛋白質の細胞壁への輸送過程に影響を与えたと判断される。

(2).真菌細胞壁の表層糖蛋白質と反応する抗体を用いる方法

被検試料がGPIアンカー蛋白質の真菌表層での発現に影響を与えるか否かは、真菌細胞壁中のGPIアンカー蛋白質を、該蛋白質と反応する抗体によって定量することにより検出が可能である。

抗体としては、例えばGPIアンカー蛋白質例えばα-agglutinin・Cwp2p・Als1p等のアミノ酸配列より抗原決定基を予想して（Chen MH et al,

J. Biol. Chem., 270:26168-26177, 1995, Van Der Vaat JM et al, J. Bacteriol., 177:3104-3110,1995, Hoyer LL et al, Mol. Microbiol., 15:39-54, 1995)、その領域のペプチドを合成し、抗原性のある物質例えば異種蛋白質等に結合させて、家兎等に免疫してポリクローナル抗体を、マウス等に免疫してモノクローナル抗体を得ることが可能である。また、好ましくは、Als1pペプチドに対する家兎ポリクローナル抗体が望ましい。

また別法として真菌、好ましくはGPIアンカー蛋白質例えばα-agglutinin・ Cwp2p・Als1p等を過剰発現させた真菌を、場合によっては更に部分精製したGPIアンカー蛋白質を、マウス等に免疫し、融合後得られたクローンを、その産生する抗体をELISA・Western blot解析等で選択することにより、GPIアンカー蛋白質に対するモノクローナル抗体を得ることが可能である。

被検試料がGPIアンカー蛋白質の細胞壁への輸送過程に影響を与え、細胞壁中のGPIアンカー由来蛋白質の量を減少させるか否かは、以下の方法により検定できる。

真菌を、被検試料の存在下、適当な条件、例えば30℃、48時間培養する。培養した真菌を遠心により集菌し、菌体を好ましくはガラスビーズを用いて破砕する。洗浄した破砕菌体を、好ましくはSDSで抽出遠心後、沈殿を洗浄する。抽出後の破砕菌体を、グルカンを分解する酵素、好ましくはグルカナーゼで処理し、その遠心上清をGPIアンカー蛋白質サンプルとする。

抗Als1pペプチド抗体を、96 wellプレートに4℃、overnightでコーティングする。洗浄液好ましくは0.05% Tween 20含有PBS(PBST)で洗浄後、96 wellプレートの非特異的吸着部位をブロックする試薬、好ましくはBSA・ゼラチン等の蛋白質、更に好ましくはブロックエースでブロッキン

グする。再度洗浄液好ましくはPBSTで洗浄後、場合によっては適当に希釈したGPIアンカー蛋白質サンプルを加え、適当な時間例えば室温で2時間反応させる。洗浄液好ましくはPBSTで洗浄後、酵素標識したC. albicansに対する抗体、好ましくはHRP標識抗カンジダ抗体を、適当な時間例えば室温で2時間反応させる。標識の方法は酵素標識であっても、放射性同位元素による標識であっても許される。洗浄液好ましくはPBSTで洗浄後、標識に適した方法、酵素標識であれば基質溶液を加え、反応停止後490 nmの吸光度を測定することにより、GPIアンカー蛋白質サンプル中のAls1p量を算出する。

(3).動物細胞に対する付着能により検定する方法

被検試料がGPIアンカー蛋白質の真菌表層での発現に影響を与えるか否かは、真菌細胞壁中のGPIアンカー蛋白質の活性、好ましくは真菌の動物細胞への付着能等を測定することにより、検定が可能である。GPIアンカー蛋白質の活性としては、動物細胞への付着に関与するAls1p、Hwp1等の他に、matingに関与するα-agglutinin、酵母の凝集に関与するFlo1p等が知られている。以下に、真菌の動物細胞への付着能により検定する方法について具体的に記載するが、本発明はこれにより限定されるものではない。

真菌としては、細胞に対する付着能を有する真菌を使用し、好ましくは真菌はC. albicansであることが望ましい。哺乳類細胞としては真菌が接着する性質を有する細胞を使用し、好ましくは細胞は腸管上皮細胞であることが望ましい。哺乳類細胞を培養し、適当な方法例えばエタノール固定により固定する。そこへ被検試料と適当な時間、例えば30℃で48時間インキュベートした真菌を接種し、一定時間例えば30℃で1時間培養後、培養上清を除去しバッファーで洗浄して寒天培地、例えばサブロー・デキストロース寒天培地（Difco）を重層する。30℃一晩培養後、コ

ロニー数をカウントし、付着率を計算する。

被検試料に、化合物処理を行わなかった真菌と比較して、細胞に付着することにより形成されたコロニー数を低下させる活性が認められれば、該被検試料はGPIアンカー蛋白質の細胞壁への輸送過程に影響を与えたと判断される。

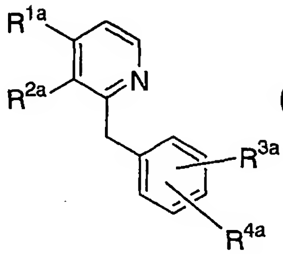
(4).真菌を電子顕微鏡あるいは光学顕微鏡で観察する方法

被検試料がGPIアンカー蛋白質の真菌表層での発現に影響を与えるか否かは、真菌細胞壁の構造を電子顕微鏡により観察することにより検定が可能である。

被検試料の存在下で、真菌例えばC. albicansを、一定時間例えば30℃で48時間培養し、透過型電子顕微鏡を用いて超微形態学的構造を観察する。ここで、透過型電子顕微鏡による観察は、例えば電子顕微鏡チャートマニュアル（医学出版センター）に記載の方法により行うことができる。透過型電子顕微鏡像で見られる、電子密度の高い菌体最外層の綿状線維構造は、GPIアンカー蛋白質を構成成分とする表層糖蛋白質層であると考えられ、既存の他の抗真菌剤では影響を受けない。無処置菌体と比較し、この電子密度の高い菌体最外層の綿状線維構造が、僅かな高電子密度の層を残して消失している場合は、該被検試料が、GPIアンカー蛋白質の細胞壁への輸送過程に影響を与えたと判断される。

また透過型電子顕微鏡に併せ、光学顕微鏡下による観察で、真菌細胞が大きく膨化し出芽（分裂）が阻害されている像が観察される場合、該被検試料が細胞壁に対して影響を与えていると判断される。

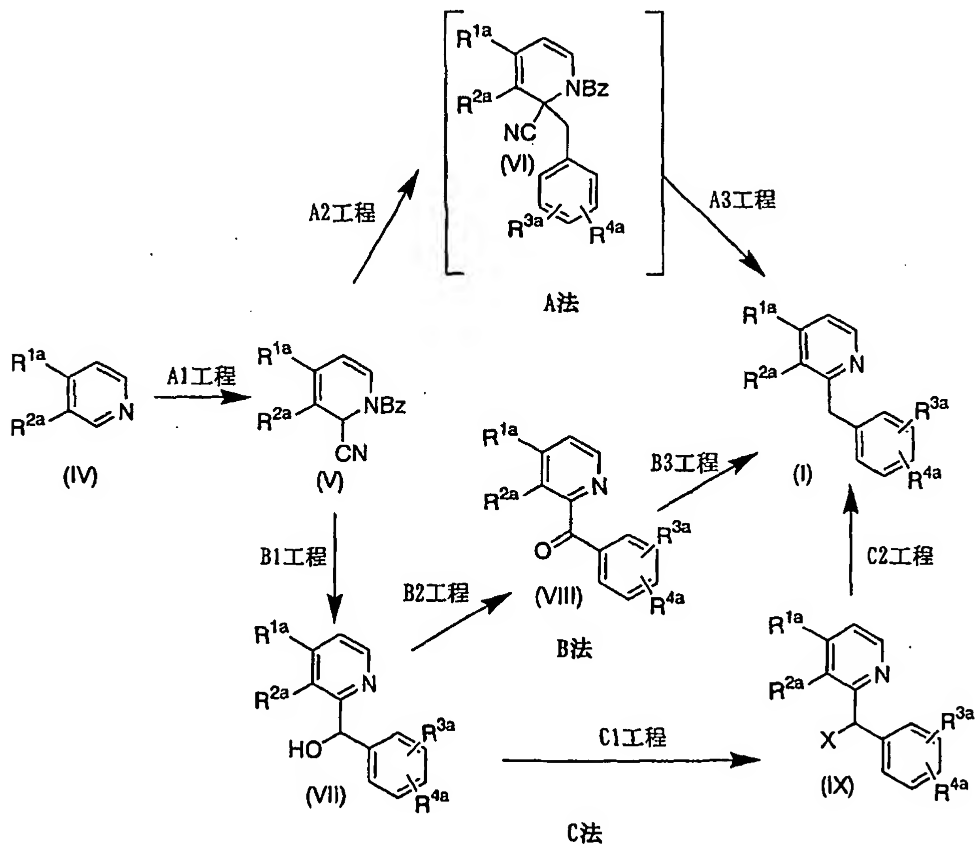
式(I)



(I)

（式中の記号は前記定義に同意義を意味する。）で表わされる本発明化合物は、これまでに知られている通常の有機化学反応などを利用して合成することができるが、例えば以下の方法で合成することができる。

一般製造方法（１）



（式中、Xはハロゲン基、アシル基などの脱離基を表す。Ｒ３ｃは、Ｒ３ａと同意義を示す。式中のその他の記号は前記定義と同意義を意味する。）

A1工程　　ライセルト(Reissert) 化合物 (V) を製造する反応である。Org. Synth., VI, 115(1988)、Heterocycles, 36(11), 2489(1993)、J. Chem. Soc. (C), 666(1969)、またはJ. Heterocycl. Chem., 29(5),

1165(1992)などの文献に記載の反応条件に基づいて製造することができる。用いる試薬としては具体的には、例えばベンゾイルクロリドとシアン化カリウムの組み合わせの条件等があげられる。

A2工程　アルキル化の工程である。化合物(V)と置換基を有するベンジルハライド誘導体や置換基を有するベンジルメタンスルフォナート誘導体などと塩基存在下反応させることにより化合物(VI)を製造することができる。塩基としては具体的には、例えば水素化ナトリウム、水酸化ナトリウムなどを挙げることができる。

A3工程　加水分解反応の工程である。化合物(VI)を塩基存在下、加水分解することにより化合物(I)を製造することができる。

A法とは、A1工程、A2工程そしてA3工程を経由して化合物(I)を製造する方法である。

B1工程　化合物(V)から化合物(VII)への工程である。化合物(V)と置換基を有するベンズアルデヒドを塩基と相間移動触媒の存在下、反応させることにより化合物(VII)を製造することができる。例えば、塩基としては、水酸化ナトリウム、水酸化カリウムなどが挙げられる。相間移動触媒としては、トリエチルベンジルアンモニウムクロリドなどが挙げられる。

B2工程　アルコールからケトンへの酸化の工程である。アルコールからケトンへの酸化反応で一般に用いられる酸化剤、条件を用いることによりケトン体(VIII)を製造することができる。酸化剤としては具体的には、例えば二酸化マンガン、二酸化クロムまたはベンゾキノンなどが挙げられる。

B3工程　ケトンからメチレンへの還元の工程である。ケトン体(VIII)からメチレン体(I)への還元反応で一般に用いられる還元剤の条件を用いることによりメチレン体(I)を製造することができる。例えば、還元剤

としては、ヒドラジン水和物と水酸化ナトリウムあるいは水酸化カリウム、トリエチルシランとボロントリフルオライドあるいはトリフルオロメタンスルフォン酸などが挙げられる。

B法とは、A1工程、B1工程、B2工程そしてB3工程を経由して化合物(I)を製造する方法である。

C1工程　水酸基のハロゲン化あるいはアシル化の工程である。化合物(VII)をハロゲン化剤あるいはアシル化剤を用いて化合物(IX)を製造することができる。ハロゲン化剤としては、例えば塩化チオニル、濃塩酸、三臭化リンなどがあげられる。また、アシル化剤としては、例えばアセチルクロリドなどの酸ハライド、無水酢酸などの酸無水物などが挙げられる。

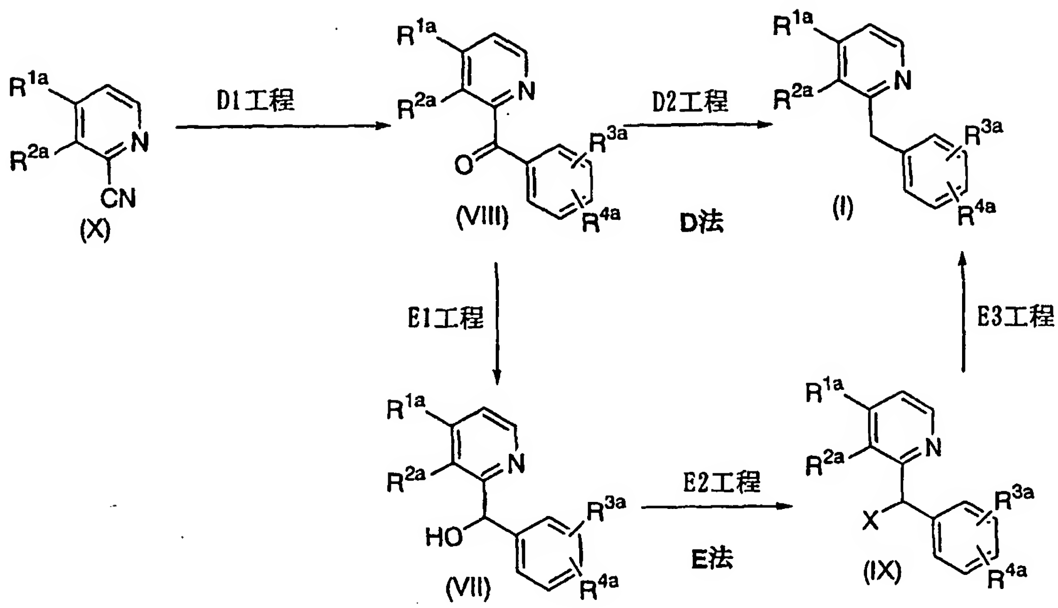
C2工程　ハロゲン基あるいはアシル基の還元的脱離反応の工程である。化合物(IX)を触媒などを用いて水素化脱離することにより化合物(I)を製造することができる。

例えば、触媒としては、パラジウムー炭素などが挙げられる。

C法とは、A1工程、B1工程、C1工程そしてC2工程を経由して化合物(I)を製造する方法である。

一般製造方法（２）

一般式(Ｉ)で表わされる本発明化合物は、以下の方法でも合成することができる。



(式中、Xはハロゲン基、アシル基などの脱離基を表す。式中のその他の記号は前記定義と同意義を意味する。)

D1工程　グリニャール反応とそれに続く酸加水分解反応の工程である。化合物(X)と置換基を有していてもよいフェニルグリニャール試薬を反応させ、続いて酸存在下加水分解することにより化合物(VIII)を製造することができる。

D2工程　B3工程と同様な条件により、ケトン体(VIII)からメチレン体(I)を製造することができる。

D法とは、D1工程とD2工程を経由して化合物(I)を製造する方法である。

E1工程　ケトンからアルコールへの還元反応の工程である。ケトンからアルコールへの還元反応で一般に用いられる還元剤、条件を用いて化合物(VIII)から化合物(VII)を製造することができる。用いる還元剤としては具体的には、例えば水素化ホウ素ナトリウム、水素化アルミニウムリチウムなどが挙げられる。

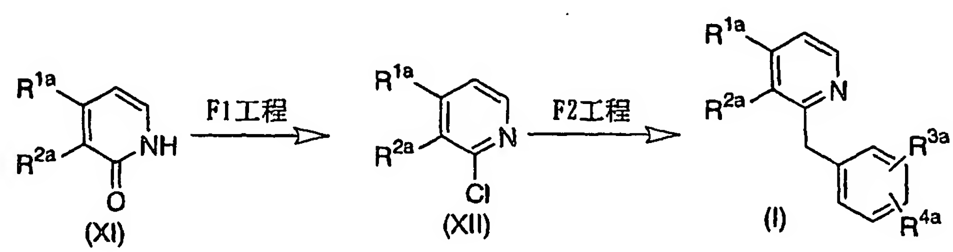
E2工程　C1工程と同様な条件により、アルコール体(VII)からハロゲン化あるいはアシル化体(IX)を製造することができる。

E3工程　C2工程と同様な還元的脱離反応の条件で、化合物(IX)から化合物(I)を製造することができる。

E法とは、D1工程、E1工程、E2工程そしてE3工程を経由して化合物(I)を製造する方法である。

一般製造方法（３）

一般式(Ｉ)で表わされる本発明化合物は、以下の方法でも合成することができる。



F法

(式中の記号は前記定義に同意義を意味する。)

F1工程　塩素化反応の工程である。化合物(XI)を塩素化剤用いることにより化合物(XII)を製造することができる。塩素化剤としては、例えばオキシ塩化リン、塩化チオニルなどが挙げられる。

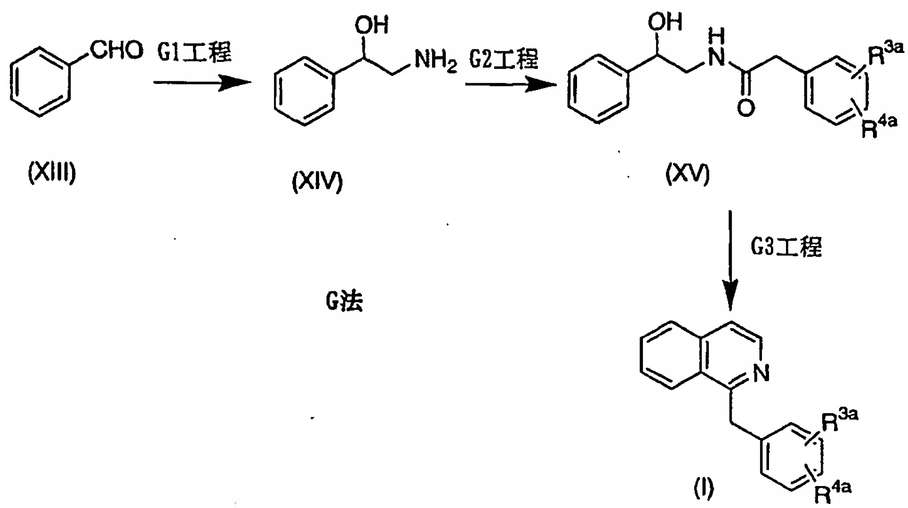
F2工程　グリニャール試薬とのカップリング反応の工程である。Arch. Pharm, 314, 156(1981)などの文献に記載の反応条件に基づいて、化合物(XII)に置換基を有していても良いベンジルグリニャール試薬を触媒存在下反応させることにより化合物(I)を製造することができる。触媒としては、例えば、[1,1'-ビス(ジフェニルホスフィノ)フェロセン]ジクロロニッケル(II)などが挙げられる。

F法とは、F1工程とF2工程を経由して化合物(I)を製造する方法である。

一般製造方法（４）

本発明化合物、一般式(Ｉ)のうち、R^{1a}とR^{2a}が一緒になってベンゼン環、

ピリジン環、ピロール環、チオフェン環、フラン環、シクロヘキサン環、またはシクロペンタン環などの縮合環を形成する場合、以下の方法で合成することができる。



(式中の記号は前記定義に同意義を意味する。)

製造方法の例としてイソキノリン環を形成する場合の製造方法を示す。

G1工程　縮合反応とそれに続く還元反応の工程である。置換基を有していてもよいベンズアルデヒド誘導体(XIII)とニトロメタンとの縮合反応後、ニトロ基の還元を行うことにより化合物(XIV)を製造することができる。例えば、ニトロ基の還元に用いられる試薬としては、パラジウムー炭素とギ酸アンモニウム、水素化アルミニウムリチウムなどの組み合わせが挙げられる。

G2工程　アミド結合形成反応である。化合物(XIV)と置換基を有していても良いフェニル酢酸クロリドをアミド結合生成反応に用いるカップリング試薬を用いることにより化合物(XV)を製造することができる。例えば、*N*,*N*'-ジシクロヘキシルカルボジイミドと*N*-ヒドロキシスクシンイミド、*N*,*N*'-ジシクロヘキシルカルボジイミドと*N*-ヒドロキシベンゾトリアゾール、1,1'-カルボニルジイミダゾールなどが挙げられる。

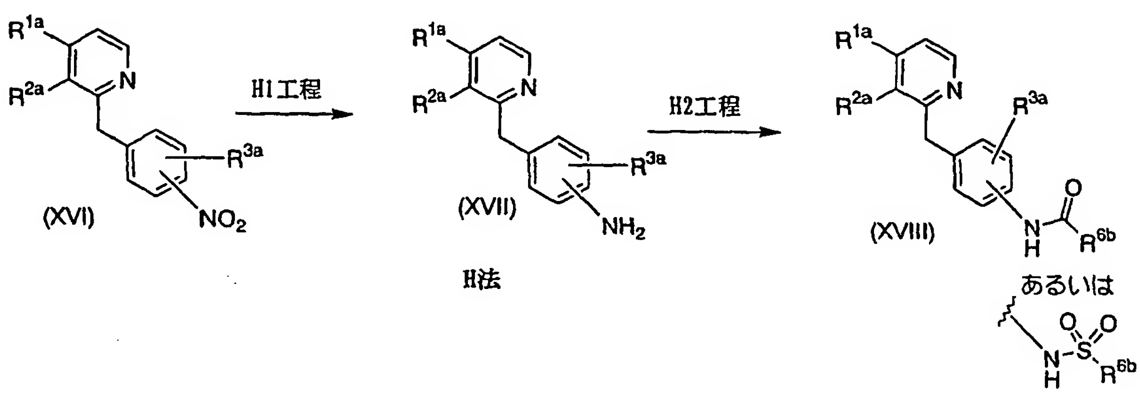
G3工程　環化反応の工程である。化合物(XV)をOrganic Reaction, 6, 74(1951)、J. Hetetocyclic Chem., 30, 1581(1993)などの文献に記載の反応条件に基づいて、製造することができる。例えば、試薬としてはオキシ塩化リン、ポリリン酸などが挙げられる。

G法とは、G1工程、G2工程そしてG3工程を経由して化合物(I)を製造する方法である。

一般製造方法（５－１）

前記の一般製造方法で合成した化合物(Ｉ)のR^{3a}、R^{4a}の置換基変換

（５－１）アミノ基、アミド基、スルホンアミド基等への置換基の変換



H法

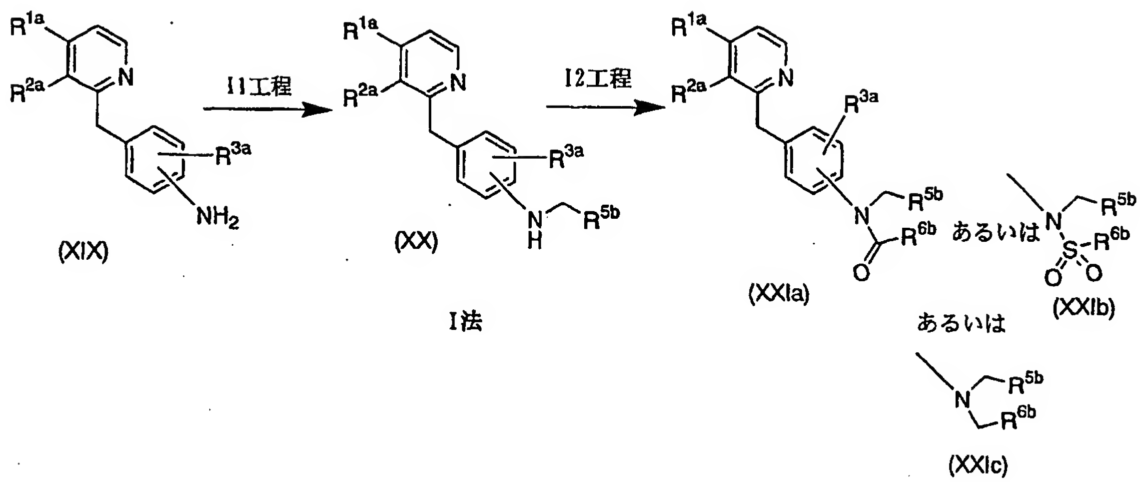
(式中の記号は前記定義に同意義を意味する。)

H1工程　ニトロ基の還元反応である。化合物(XVI)を一般的に利用されるニトロ基の還元法で還元することにより化合物(XVII)を製造することができる。例えば、ニトロ基の還元法としては、パラジウム－炭素、水酸化パラジウムよる接触水素化還元、鉄－塩化アンモニウム、鉄－塩酸、鉄－酢酸などによる還元が挙げられる。

H2工程　アシル化あるいはスルフォニル化反応の工程である。化合物(XVII)を酸クロリドあるいは酸無水物を用いることにより化合物(XVIII)を製造することができる。

H法とは、H1工程とH2工程を経由して化合物(XVIII)を製造する方法で

ある。



(式中の記号は前記定義に同意義を意味する。)

I1工程　還元的アミノ化反応の工程である。化合物(XIX)と置換基を有していても良いアルデヒドをJ. Am. Chem. Soc., 93, 2897(1971)、Comprehensive Organic Synthese, 8, 25(1991)、Tetrahedron, 40, 1783(1984)そしてTetrahedron, 41, 5307(1985)などの文献に記載の反応条件に基づいて、化合物(XX)を製造することができる。例えば、還元的アミノ化試薬としては、トリアセトキシ水素化ホウ素ナトリウム、シアン水素化ホウ素ナトリウム、ボラン-ピリジン錯体、パラジウム-炭素／水素等が挙げられる。

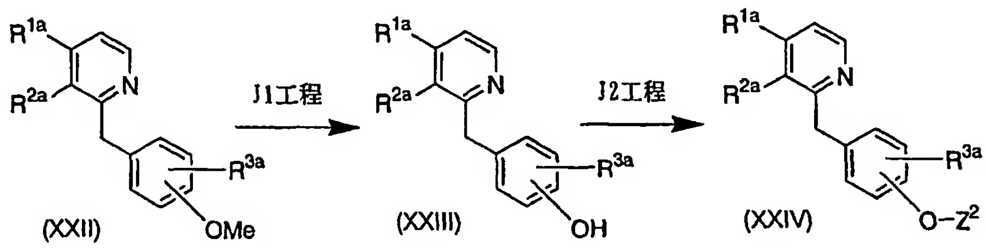
I2工程　アシル化、スルフォニル化あるいは還元的アミノ化反応の工程である。化合物(XX)を酸クロリドあるいは酸無水物を用いることにより化合物(XXIa)あるいは化合物(XXIb)を製造することができる。または、還元的アミノ化反応をI1工程と同様に行うことにより化合物(XXIc)を製造することができる。

I法とは、I1工程とI2工程を経由することにより化合物(XXIa)、化合物(XXIb)あるいは化合物(XXIc)を製造する方法である。

一般製造方法（５－２）

前記の一般製造方法で合成した化合物(I)のR^{3a}、R^{4a}の置換基変換

(5-2) 水酸基、アルコキシ基等への置換基の変換



J法

(式中の記号は前記定義に同意義を意味する。)

J1工程　脱メチル化反応で、Bull. Chem. Soc. Jpn., 44, 1986(1971)、Org. Synth., Collect. Vol. V, 412(1073)、J. Am. Chem. Soc., 78, 1380(1956)、またはJ. Org. Chem., 42, 2761(1977)などの文献に記載の反応条件に基づいて、化合物(XXII)から化合物(XXIII)を製造することができる。例えば、脱メチル化反応に使用される試薬としては、47%臭化水素酸水溶液、ボロントリブロミド、ピリジン塩酸塩そしてヨードトリメチルシランなどが挙げられる。

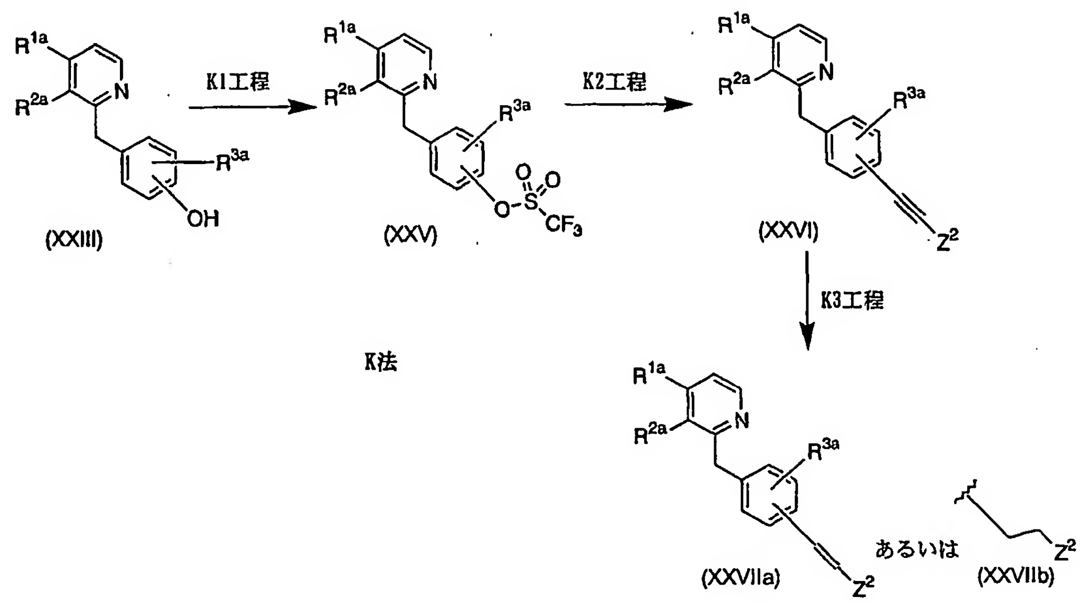
J2工程　アルキル化反応の工程である。化合物(XXIII)を塩基存在下置換基されていても良いアルキルハライドあるいは置換基されていてもよいアルキルメタンスルフォネートなどと反応させることにより化合物(XXIV)を製造することができる。

J法とは、J1工程とJ2工程を経由して化合物(XXIV)を製造する方法である。

一般製造方法(5-3)

前記の一般製造方法で合成した化合物(I)のR^{3a}、R^{4a}の置換基変換

(5-3) ビニレン基またはエチニレン基、アルキル基等への置換基の変換



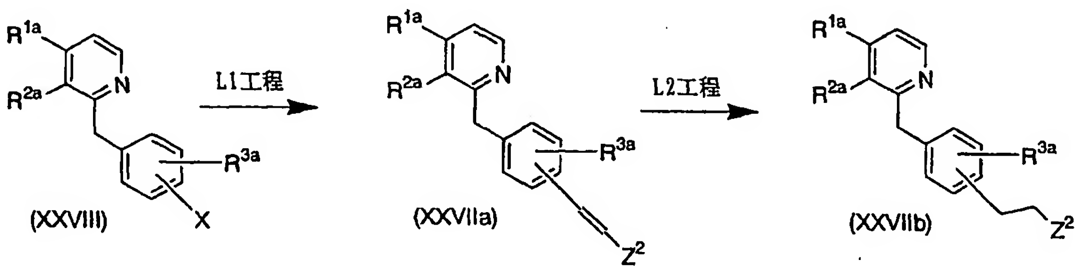
(式中の記号は前記定義に同意義を意味する。)

K1工程　トリフラート化反応の工程である。化合物(XXIII)を塩基存在下トリフルオロメタンスルフォン酸無水物と反応させることにより化合物(XXV)を製造することができる。

K2工程　アルキンとのカップリング反応の工程である。化合物(XXV)とアルキン誘導体をパラジウムのホスフィン錯体、ヨウ化銅そして塩基存在下、カップリングすることにより化合物(XXVI)を製造することができる。例えば、パラジウムのホスフィン錯体を系中で生成させる試薬としては、パラジウム－炭素とトリフェニルホスフィン、テトラキストリフェニルホスフィンパラジウム(0)とトリフェニルホスフィン、ジクロロビストリフェニルホスフィンパラジウム(II)、酢酸パラジウム(II)とトリ(o-トリル)ホスフィン、酢酸パラジウム(II)と1,1'-ビス(ジフェニルフォスフィノ)フェロセンなどが挙げられる。塩基としては、トリエチルアミン、ピペリジン、ピリジン、炭酸カリウムなどが挙げられる。反応により塩化リチウムを使用することがある。

K3工程　不飽和炭化水素の還元反応の工程である。化合物(XXVI)を触

媒を用いた接触水素化還元などにより化合物(XXVIIa)あるいは化合物(XXVIIb)を製造する方法である。例えば、触媒として用いられるものとしてはパラジウム－炭素、水酸化パラジウム、酸化白金、パラジウム－炭素－炭酸カルシウムなどが挙げられる。



Xはハロゲン原子、トリフルオロスルホネートなどの脱離基を表す。

L法

(式中の記号は前記定義に同意義を意味する。)

L1工程　アルケンとのカップリング反応（ヘック（Heck）反応）の工程である。J. Org. Chem., 37, 2320(1972)、Org. Reactions., 27, 345(1982)、Comprehensive Organic Synthesis, Vol. 4, 833(1991)、Palladium Reagents and Catalysts, 125(1995)、Chem. Commun., 1287(1984)、Tetrahedron Lett, 26, 2667(1985)そしてTetrahedron Lett, 31, 2463(1990)などの文献に記載の反応条件に基づいて、触媒（パラジウム錯体と配位子など）を用いて、化合物(XXVIII)から化合物(XXVIIa)を製造することができる。この反応に用いる触媒（パラジウム錯体と配位子）の組み合わせとしては、例えば酢酸パラジウム(II)と1,1'-ビス(ジフェニルフォスフィノ)フェロセン、酢酸パラジウム(II)とトリ(o-トリル)フォスフィンなどが挙げられる。用いられる3級塩基としてはトリエチルアミン、ジイソプロピルエチルアミンそして1,8-ジアザビシクロ[5.4.0]-7-ウンデセンなどが挙げられる。化合物(XXVIII)のXは脱離基を意味し、例えばハロゲン基、トリフルオロメタンスルフォニルオキシ

基などを挙げることができる。

L2工程　K3工程と同様な不飽和炭化水素の還元反応の条件により、化合物(XXVIIa)から化合物(XXVIIb)を製造することができる。

L法とは、L1工程により化合物(XXVIIa)、続いてL2工程により化合物(XXVIIb)を製造する方法である。

本発明にかかる前記式(I)で表わされる化合物について得られる種々の異性体は、通常の分離手段（例えば再結晶、クロマトグラフィー等）を用いることにより精製し、単離することができる。

本発明にかかる化合物もしくはその塩またはそれらの水和物は、それ自体を哺乳動物（好ましくはヒト）に投与することもできるが、慣用されている方法により錠剤、散剤、細粒剤、顆粒剤、被覆錠剤、カプセル剤、シロップ剤、トローチ剤、吸入剤、坐剤、注射剤、軟膏剤、眼軟膏剤、点眼剤、点鼻剤、点耳剤、パップ剤、ローション剤等として製剤化して投与することもできる。製剤化には通常用いられる製剤化助剤（例えば賦形剤、結合剤、滑沢剤、着色剤、矯味矯臭剤や、および必要により安定化剤、乳化剤、吸収促進剤、界面活性剤、ｐＨ調製剤、防腐剤、抗酸化剤など）を使用することができ、一般に医薬品製剤の原料として用いられる成分を配合して常法により製剤化される。例えば経口製剤を製造するには、本発明にかかる化合物またはその薬理学的に許容される塩と賦形剤、さらに必要に応じて結合剤、崩壊剤、滑沢剤、着色剤、矯味矯臭剤などを加えた後、常法により散剤、細粒剤、顆粒剤、錠剤、被覆錠剤、カプセル剤等とする。これらの成分としては例えば、大豆油、牛脂、合成グリセライド等の動植物油；流動パラフィン、スクワラン、固形パラフィン等の炭化水素；ミリスチン酸オクチルドデシル、ミリスチン酸イソプロピル等のエステル油；セトステアリルアルコール、ベヘニルアルコール等の高級アルコール；シリコン樹脂；シリコン油；ポリ

オキシエチレン脂肪酸エステル、ソルビタン脂肪酸エステル、グリセリン脂肪酸エステル、ポリオキシエチレンソルビタン脂肪酸エステル、ポリオキシエチレン硬化ひまし油、ポリオキシエチレンポリオキシプロピレンブロックコポリマー等の界面活性剤;ヒドロキシエチルセルロース、ポリアクリル酸、カルボキシビニルポリマー、ポリエチレングリコール、ポリビニルピロリドン、メチルセルロースなどの水溶性高分子;エタノール、イソプロパノールなどの低級アルコール;グリセリン、プロピレングリコール、ジプロピレングリコール、ソルビトールなどの多価アルコール;グルコース、ショ糖などの糖;無水ケイ酸、ケイ酸アルミニウムマグネシウム、ケイ酸アルミニウムなどの無機粉体、精製水などがあげられる。賦形剤としては、例えば乳糖、コーンスターチ、白糖、ブドウ糖、マンニトール、ソルビット、結晶セルロース、二酸化ケイ素などが、結合剤としては、例えばポリビニルアルコール、ポリビニルエーテル、メチルセルロース、エチルセルロース、アラビアゴム、トラガント、ゼラチン、シェラック、ヒドロキシプロピルメチルセルロース、ヒドロキシプロピルセルロース、ポリビニルピロリドン、ポリプロピレングリコール・ポリオキシエチレン・ブロックポリマー、メグルミンなどが、崩壊剤としては、例えば澱粉、寒天、ゼラチン末、結晶セルロース、炭酸カルシウム、炭酸水素ナトリウム、クエン酸カルシウム、デキストリン、ペクチン、カルボキシメチルセルロース・カルシウム等が、滑沢剤としては、例えばステアリン酸マグネシウム、タルク、ポリエチレングリコール、シリカ、硬化植物油等が、着色剤としては医薬品に添加することが許可されているものが、矯味矯臭剤としては、ココア末、ハッカ脳、芳香散、ハッカ油、竜脳、桂皮末等が用いられる。これらの錠剤・顆粒剤には糖衣、その他必要により適宜コーティングすることはもちろん差支えない。また、シロップ剤や注射用製剤等の液剤を製造する際に

は、本発明にかかる化合物またはその薬理学的に許容される塩にpH調整剤、溶解剤、等張化剤などと、必要に応じて溶解補助剤、安定化剤などを加えて、常法により製剤化する。外用剤を製造する際の方法は限定されず、常法により製造することができる。すなわち製剤化にあたり使用する基剤原料としては、医薬品、医薬部外品、化粧品等に通常使用される各種原料を用いることが可能である。使用する基剤原料として具体的には、例えば動植物油、鉱物油、エステル油、ワックス類、高級アルコール類、脂肪酸類、シリコン油、界面活性剤、リン脂質類、アルコール類、多価アルコール類、水溶性高分子類、粘土鉱物類、精製水などの原料が挙げられ、さらに必要に応じ、pH調整剤、抗酸化剤、キレート剤、防腐防黴剤、着色料、香料などを添加することができるが、本発明にかかる外用剤の基剤原料はこれらに限定されない。また必要に応じて分化誘導作用を有する成分、血流促進剤、殺菌剤、消炎剤、細胞賦活剤、ビタミン類、アミノ酸、保湿剤、角質溶解剤等の成分を配合することもできる。なお上記基剤原料の添加量は、通常外用剤の製造にあたり設定される濃度になる量である。

本発明にかかる化合物もしくはその塩またはそれらの水和物を投与する場合、その形態は特に限定されず、通常用いられる方法により経口投与でも非経口投与でもよい。例えば錠剤、散剤、顆粒剤、カプセル剤、シロップ剤、トローチ剤、吸入剤、坐剤、注射剤、軟膏剤、眼軟膏剤、点眼剤、点鼻剤、点耳剤、パップ剤、ローション剤などの剤として製剤化し、投与することができる。本発明にかかる医薬の投与量は、症状の程度、年齢、性別、体重、投与形態・塩の種類、疾患の具体的な種類等に応じて適宜選ぶことができる。

本発明にかかる抗真菌剤は、患者に対して治効量投与される。ここで「治効量」とは、意図される薬理学的結果を生じさせ、処置されるべき

患者の症状を回復または軽減するために有効な薬剤の量である。投与量は、患者の体重、疾患の種類、症状の程度、患者の年齢、性差、薬剤に対する感受性差などにより著しく異なるが、通常成人として１日あたり、約０．０３－１０００mg、好ましくは０．１－５００mg、さらに好ましくは０．１－１００mgを１日１－数回、または数日に１－数回に分けて投与する。注射剤の場合は、通常約１μｇ／kg－３０００μｇ／kgであり、好ましくは約３μｇ／kg－１０００μｇ／kgである。

図面の簡単な説明

図１は、GPIアンカー蛋白質の細胞壁への輸送過程の模式図。GPIアンカー蛋白質は、一旦GPI（Glycosylphosphatidylinositol）にアンカーした後、細胞壁に輸送される。

図２は、S. cerevisiaeレポータ系での前記式（Ｉａ）に記載の化合物の活性を示すグラフ。前記式（Ｉａ）に記載の化合物の存在下では、0.39～1.56μg/mlの濃度で培養上清画分中のセファロスポリナーゼ活性が上昇、細胞壁画分中の活性が低下し、3.13μg/ml以上の濃度で増殖抑制が見られた。

図３は、C. albicansの動物細胞付着への前記式（Ｉａ）に記載の化合物の影響を示すグラフ。増殖抑制の見られない1.56μg/mlの濃度でも、C. albicansの動物細胞への付着が半分程度にまで抑制された。

図４は、C. albicansのAls1p抗原量への前記式（Ｉａ）に記載の化合物の影響を示すグラフ。前記式（Ｉａ）に記載の化合物の存在下では、0.1～0.39μg/mlの濃度で、培養上清画分中のAls1p抗原量が上昇し、細胞壁画分中の抗原量が低下した。

図５は、C. albicans遺伝子のGWT1遺伝子をプローブとしたサザンブロット解析を示す写真。EcoRIで6.5 kb、HindIIIで4.0 kb、EcoRI-Hind

IIIで2.0 kb、EcoRI-PstIで2.5 kbの単一のバンドが観察され、C. albicansの前記式（Ｉａ）に記載の化合物に対する耐性遺伝子のホモログは、単一の遺伝子として存在することが予想された。

図６は、GWT1遺伝子産物を過剰発現したS. cerevisiaeにおける前記式（Ｉａ）に記載の化合物の活性を示すグラフ。S. cerevisiae CW63株（図中の「W/T」）では、培養上清画分中のセファロスポリナーゼ活性が上昇し、細胞壁画分中の活性が低下している前記式（Ｉａ）に記載の化合物濃度（0.39～1.56μg/ml）でも、S. cerevisiae CW63/GWT1株では影響が見られず、またS. cerevisiae CW63株では増殖が抑制される前記式（Ｉａ）に記載の化合物濃度（> 3.13μg/ml）でも、S. cerevisiae CW63/GWT1株（図中の「O/E」）では増殖抑制が見られなかった。

図７は、S.cerevisiae, S.pombe, C.albicansのGWT1遺伝子のコードする蛋白において高度に保存されている領域を整列させた図。

発明を実施するための最良の形態

[実施例Ａ]

以下の実施例を挙げて本発明をより具体的に説明するが、これらは本発明の範囲を制限するものではない。

実施例Ａ１　レポータ遺伝子の構築とS. cerevisiaeへの導入

(1).リゾチームをレポータ酵素とするレポータ遺伝子の構築

ENO1プロモーター＋分泌シグナル＋リゾチーム遺伝子を含むプラスミドpESH（Ichikawa K et al, Biosci. Biotech. Biochem., 57(10), 1686-1690, 1993）を鋳型に、配列番号８及び配列番号９に記載のオリゴヌクレオチドをプライマーとして、プロモータ配列を含むリゾチーム遺伝子をPCRにより増幅し、pCR-Script SK(+)のSalI-EcoRI siteにサブクローニングした(a)。また、S. cerevisiae染色体DNAを鋳型に、配列番

号１０及び配列番号１１に記載のオリゴヌクレオチドをプライマーとしてCWP2遺伝子をPCR増幅し、pUC19のEcoRI-HindIII siteにサブクローニングした(b)。同様に、pYES2 (INVITROGEN) を鋳型に、配列番号１２及び配列番号１３に記載のオリゴヌクレオチドをプライマーとしてしてCYC1ターミネーターをPCR増幅し、pUC19の新たに導入したNotI-KpnI site サブクローニングした(c)。

次に、pESHのSalI-HindIII切断部分にSalI-EcoRIで切り出したリゾチーム遺伝子(a)およびEcoRI-HindIIIで切り出したCWP2遺伝子(b)を挿入した。最後に、ENO1プロモーター＋分泌シグナル＋リゾチーム遺伝子＋CWP2遺伝子を含む遺伝子をBamHI-HindIIIで切り出し、インテグレーション用ベクターpRS306 (Sikorski RS et al, Genetics. 122(1):19-27, 1989) に挿入後、HindIII-KpnI切断部分にHindIII-KpnIで切り出したCYC1ターミネーター(c)を挿入し、pRLW63Tを作製した。

(2).セファロスポリナーゼをレポータ酵素とするレポータ遺伝子の構築

上述のpESHを鋳型にして、ENO1プロモーターＣ末＋分泌シグナル部分(d)を鋳型にし、配列番号１４及び配列番号１５に記載のオリゴヌクレオチドをプライマーとして、プロモータ配列・分泌シグナル部分を含むDNAをPCRにより増幅し、pUC19の新たに導入したBamHI-NotI siteににサブクローニングした(d)。また、Citrobacter freundii染色体DNAを鋳型にし、配列番号１６及び配列番号１７に記載のオリゴヌクレオチドをプライマーとして、セファロスポリナーゼ遺伝子をPCR増幅し、pUC19の新たに導入したNspV-XbaI siteにサブクローニングした(e)。同様にS. cerevisiae染色体DNAを鋳型にし、配列番号１８及び配列番号１９に記載のオリゴヌクレオチドをプライマーとして、CWP2遺伝子PCR増幅し、pUC19のXbaI-HindIII siteにサブクローニングした(f)。

(d)を挿入したプラスミドのBamHI-SalI切断部分にpESHのBamHI-SalI

断片を挿入し、ENO1プロモーター全長＋分泌シグナル部分を作製後、NspV-HindIII切断部分にNspV-XbaIで切り出したセファロスポリナーゼ遺伝子およびXbaI-HindIIIで切り出したCWP2遺伝子を挿入した。次いで、EcoRI-HindIIIで切り出し、上述のpRS306に挿入後、HindIII-KpnI切断部分にCYC1ターミネーターを挿入して、pRCW63Tを作製した。

(3).レポータ遺伝子のS. cerevisiaeへの導入

S. cerevisiae G2-10株を、10 mlのYPD培地にて30℃で振とう培養し、対数増殖後期(2～5 x 10^7 cells/ml)の時点で集菌した。滅菌水で洗浄後、YEASTMAKER™ Yeast Transformation System(Clontech)を用いた酢酸リチウム法（YEASTMAKER™ Yeast Transformation System User Manualに記載）によって上述したpRLW63TおよびをpRCW63Tを導入した。pRLW63TはEcoRVで、pRCW63TはApaIでURA3遺伝子を切断したものを用いた。SD(Ura$^-$)培地で30℃、3日間培養後、増殖したコロニーをYPD培地で培養した。

リゾチームおよびセファロスポリナーゼ活性の局在を確認したところ、両活性共に主として細胞壁に局在し、CWP2のC端配列が細胞壁への輸送シグナルとして働いていることが確認された。

実施例Ａ２　S. cerevisiaeレポータ系による薬剤のスクリーニング

リゾチームと比較して、セファロスポリナーゼの方が酵素反応の感度が良いことから、化合物のスクリーニングには、pRCW63T を導入した S. cerevisiae (S. cerevisiae CW63 株) を用いた。

YPD 液体培地に 30℃、48 時間静置培養後、YPD 液体培地で 100 倍希釈した菌液 (3～5 x 10^5 cells/ml) 75μl/well を、被検試料希釈液 25μl/well が入ったＶ底 96 well プレートに接種し、30℃で 48 時間静置培養した。プレートを遠心後、上清 25μl を 96 well 平底プレートにサンプリングし、培養上清画分とした。

沈殿した菌を懸濁し、2.4M ソルビトールで調整したザイモリエース

(生化学工業）溶液75μl/wellを加え、30℃、1時間作用させた。プレートを遠心後、上清10μlを96 well平底プレートにサンプリングし、15μlのリン酸バッファーを加え、細胞壁画分とした。

プールしたサンプルに200μMニトロセフィン溶液を加え、一定時間後にクエン酸バッファーで反応停止後、490 nmの吸光度を測定することにより、培地および細胞壁画分中のセファロスポリナーゼ活性を測定した。

また、被検試料存在下での菌の増殖は、肉眼による観察で判定した。

図２には、前記式（Ｉａ）に記載の化合物の存在下では、0.39～1.56 μg/mlの濃度で培養上清画分中のセファロスポリナーゼ活性が上昇し、細胞壁画分中の活性が低下することを示した。この様に、培養上清画分中のセファロスポリナーゼ活性を上昇させ、かつ細胞壁画分中のセファロスポリナーゼ活性を減少させる化合物を、GPIアンカー蛋白質の細胞壁への輸送過程を阻害する化合物とした。

実施例Ａ３　カンジダの動物細胞への付着を指標とした薬剤のスクリーニング

6穴マルチウェルプレートの各穴に、10%牛胎児血清および2 mMグルタミンを含むD-MEM培地（日水製薬）で1 x 10^5個/mlに調整したIEC-18細胞を、3 mlずつ分注した。該プレートを炭酸ガスインキュベータ内で37℃、3日間培養後、培養上清を除去し、エタノール固定した。

各濃度の被検試料を含有したサブロー・デキストロース液体培地で30℃・48時間培養したC. albicansを4 x 10^2個/mlに調整し、固定したIEC-18細胞を培養したプレートの各穴に、1 ml接種した。30℃・1時間培養後、培養上清を除去し、PBSで洗浄後、サブロー・デキストロース寒天培地（Difco）を2 ml重層した。30℃、一夜培養後、増殖してきたコロニー数（CFU）をカウントし、付着率を算出した。

図３には前記式（Ｉａ）に記載の化合物で、増殖抑制の見られない1.

56μg/mlの濃度でも、C. albicansの動物細胞への付着が半分程度にまで抑制されたことを示した。処理しないC.albicansと比較して、細胞に付着したCFUを減少させた被検試料を、C.albicansの動物細胞への付着を抑制する化合物とした。

実施例A4　ELISAによるGPIアンカー蛋白質の定量値を指標とした薬剤のスクリーニング

(1).抗Als1pペプチド抗体の作製

配列番号20に記載の合成ペプチドをKLHとコンジュゲートし、家兎に免疫した。得られた抗血清をアフィニティ精製し、IgG画分を抗Als1pペプチド抗体とした。

(2).抗Als1pペプチド抗体を用いたELISAによる薬剤のスクリーニング

C. albicansを、各濃度の被検薬剤を含有したサブロー・デキストロース液体培地中(5 ml)で30℃・48時間培養し、遠心による集菌、洗浄後、300μlのトリス塩酸バッファーに懸濁した。懸濁した菌体を、ガラスビーズを入れたマイクロチューブに移し、1分間の攪拌、1分間の氷冷を10回繰り返すことにより破砕した。洗浄した破砕菌体を2% SDSで95℃・10分間抽出し、遠心後、沈殿をリン酸バッファーで5回洗浄した。その沈殿に5μg/mlのザイモリエース溶液0.5 mlを加え37℃・1時間反応後、その遠心上清をGPIアンカー蛋白質サンプルとした。

50μlの抗Als1pペプチド抗体(40μg/ml)を、96 wellプレートに4℃・overnightコーティングした。0.05% Tween 20含有PBS (PBST) で5回洗浄後、25%ブロックエースで室温、2時間ブロッキングした。PBSTで3回洗浄後、2倍階段希釈したGPIアンカー蛋白質サンプル50μlを室温、2時間反応させた。PBSTで5回洗浄後、1000倍希釈したHRP標識抗カンジダ抗体(ViroStat) 100μlを室温、2時間反応させ、PBSTで5回洗浄後、基質溶液75μlを加えた。反応停止後、490 nmの吸光度を測定した。

図4には、前記式（Ｉａ）に記載の化合物の存在下では、0.1～0.39 μg/mlの濃度で、培養上清画分中のAls1p抗原量が上昇し、細胞壁画分中の抗原量が低下していることを示した。この様に、化合物で処理しないC. albicansと比較して、ELISAで定量した培養上清画分中のAls1p量細を上昇させ、あるいは胞壁画分中のAls1p量を減少させた化合物を、C.albicansのGPIアンカー蛋白質の細胞壁への輸送過程を阻害する化合物とした。

実施例Ａ５　被検試料の存在下で培養したC. albicans細胞壁の電子顕微鏡による観察

各濃度の被検薬剤を含有したサブロー・デキストロース液体培地中（5 ml）で30℃・48時間培養後、遠心、集菌したC. albicansを過マンガン酸カリ固定法により固定し、透過型電子顕微鏡像を観察した。

菌体最外層に電子密度の高い綿状線維構造が観察され、GPIアンカー蛋白質を構成成分とする表層糖蛋白質層であると考えらた。この綿状線維構造は既存の他の抗真菌剤では影響を受けなかった。

前記式（Ｉａ）に記載の化合物の存在下で培養したC. albicansは、無処置菌体と比較し、電子密度の高い菌体最外層の綿状線維構造が、僅かな高電子密度の層を残して消失していた。この様に、電子密度の高い菌体最外層の綿状線維構造が消失している場合に、被検試料をGPIアンカー蛋白質の細胞壁への輸送過程に影響を与える化合物とした。

実施例Ａ６　S. cerevisiaeの前記式（Ｉａ）に記載の化合物に対する耐性遺伝子のスクリーニング

S. cerevisiae遺伝子のプラスミドライブラリーは、ATCC(Information for ATCC Number: 37323)から入手した。

S. cerevisiae G2-10株を、10 mlのYPD培地にて30℃で振とう培養し、対数増殖後期(1～2 x 10^7 cells/ml)の時点で集菌した。滅菌水で洗浄後、YEASTMAKER™ Yeast Transformation System(Clontech)を用いた酢酸リ

チウム法（YEASTMAKER™ Yeast Transformation System User Manualに記載）によって、S. cerevisiae遺伝子のプラスミドライブラリーを導入し、SD(Leu-)プレート上にに撒いて、約80000個のコロニーを得た。コロニーを回収・希釈し、前記式（Ｉａ）に記載の化合物を 1.56μg/ml及び3.125μg/mlの濃度で含むSD(Leu⁻)プレートに、プレート当たり57万コロニーになるように撒いた。その後、37℃で72時間インキュベートして耐性クローンを獲得した。

27個のクローンをピックアップし、METHODS IN ENZYMOLOGY, Vol. 194: 169-182 (1991)に記載の方法によりプラスミドを回収して、インサートを解析したところ、27個全てが同一のフラグメントを含んでいた。

ABI377 system(PE apllied Biosystems社製)を用いて塩基配列を決定した結果、配列番号１に記載のDNAが、前記式（Ｉａ）に記載の化合物に対する耐性を付与するDNAであることが明らかとなりGWT1と命名した。

実施例Ａ７　S. cerevisiae GWT1遺伝子の、C. albicansホモログのサザンブロット解析

25μgのC. albicansゲノムDNAを、EcoRI (TaKaRa)、HindIII (TaKaRa)、BamHI (TOYOBO)、PstI (New England Biolabs)（２種類の酵素の組み合わせも含む）で16時間処理後、エタノール沈殿により濃縮し、25μlの滅菌水に溶解してサンプルとした。制限酵素消化した25μgのgenome DNAを、0.75％アガロースゲル電気泳動法により分離し、ナイロンメンブレン（GeneScreen PLUS /NEN）へトランスファーした。

プローブは、配列番号１に記載の約1.5 kbのDNAフラグメント20 ngを、ランダムプライマー法によりalpha33P-dCTPでラベルし、GeneQuantカラム（Amersham-Pharmacia）を用いて精製し作製した。

ハイブリダイゼーションは、メンブレンを、10 mlのPerfectHyb™（TOYOBO)溶液に浸し65℃で１時間プレインキュベーションをおこなった後、

ラベルした上記プローブを添加し、65℃で更に2.5時間インキュベーションした。洗浄は、1).2xSSC, 0.05% SDS溶液：25℃5分、2).2xSSC, 0.05% SDS溶液：25℃15分、3).0.1xSSC, 0.1% SDS溶液50℃20分で行った。洗浄後のメンブレンをサランラップで包み、Imaging Plate（FUJI）と室温で12時間接触させ、Imaging Plateに転写されたイメージをBAS2000(FUJI）を用いて取り込み、画像解析をおこなった。

その結果、EcoRIで6.5 kb、HindIIIで4.0 kb、EcoRI-HindIIIで2.0 kb、EcoRI-PstIで2.5 kbの単一のバンドが観察され（図５）、C. albicansの前記式（Ｉａ）に記載の化合物に対する耐性遺伝子のホモログは、単一の遺伝子として存在することが予想された。

実施例Ａ８　C. albicansの前記式（Ｉａ）に記載の化合物に対する耐性遺伝子のスクリーニング

C. albicansのゲノムライブラリーは、Navaro-Garcia F et al, Mol. Cell. Biol., 15: 2197-2206, 1995に記載の方法により作製した。具体的には、C. albicansのゲノムDNAをSau3AIで部分消化した後、３～５kb前後のDNAフラグメントを回収し、YEp352シャトルベクターのBamHIサイトに挿入した。

S. cerevisiae G2-10株を、10 mlのYPD培地にて30℃で振とう培養し、対数増殖後期(2～5 x 10^7 cells/ml)の時点で集菌した。滅菌水で洗浄後、YEASTMAKER™ Yeast Transformation System(Clontech)を用いた酢酸リチウム法（YEASTMAKER™ Yeast Transformation System User Manualに記載）によって、C. albicansのゲノムライブラリーを導入し、SD（Ura$^-$）プレート上にに撒いて、約25000個のコロニーを得た。コロニーを回収・希釈し、前記式（Ｉａ）に記載の化合物を 1.56μg/mlの濃度で含むSDプレートに、プレート当たり50万コロニーになるように撒いた。その後、30℃で６時間、37℃へ移して66時間インキュベートして耐性クロ

ーンを獲得した。

30個のクローンをピックアップし、METHODS IN ENZYMOLOGY, Vol. 194: 169-182 (1991)に記載の方法によりプラスミドを回収して、インサートを解析したところ、30個のうち28個が同一のフラグメントを含んでいた。

ABI377 system (PE apllied Biosystems社製) を用いて、塩基配列を決定した結果、配列番号３に記載のDNAが、前記式（Ｉａ）に記載の化合物に対する耐性を付与するDNAであることが明らかとなった。

実施例Ａ９　C. albicans臨床分離株からの前記式（Ｉａ）に記載の化合物に対する耐性遺伝子ホモログのクローニング

発明者らが保存するC. albicans臨床分離株より精製した、ゲノムDNAを鋳型とし､配列番号２１及び配列番号２２をプライマーとしてPCRによる増幅を行った。独立した３本のPCRサンプルから、いずれも約1.6 kbのDNAフラグメントが増幅され、増幅されたフラグメントを精製し、pT7-Blueベクター (Novagen) にサブクローニングして塩基配列を決定したところ、配列番号５に示すDNA配列が見いだされた。実施例Ａ７に記載のDNA (配列番号３) との間で３箇所の配列が異なっていた。

また、Stanford大のsequenceセンター(http://sequence-www.stanford.edu/)で決定されたC. albicans遺伝子塩基配列中にも、実施例Ａ７に記載のDNAのホモログが見出され (配列番号７) 、実施例Ａ７に記載のDNA (配列番号３) との間で４箇所の配列が異なっていた。

実施例Ａ１０　GWT1遺伝子産物を過剰発現したS. cerevisiaeの作製

実施例Ａ６で得られた前記式（Ｉａ）に記載の化合物に対する耐性クローンより精製したプラスミドを鋳型とし、配列番号２３及び配列番号２４をプライマーとして､PCR増幅を行った。PvuIIで切断したPCR産物を、実施例A1で作製したpRLW63TのSalI-HindIII切断部分に挿入した。

BamHI-KpnI でインサート全体を切り出し、pRS304 (Sikorski RS et al, Genetics. 122(1): 19-27, 1989) の MCS に挿入し、インテグレーション用ベクターを作製した。

セファロスポリナーゼ遺伝子ををレポータ遺伝子として持つ、S. cerevisiae CW63 株を実施例Ａ１に記載の方法で培養し、インテグレーション用ベクターの TRP1 を EcoRV で切断後、実施例Ａ１に記載の方法で形質転換した。SD(Trp⁻)培地で 30℃、３日間培養することにより GWT1 過剰発現株を得た（S. cerevisiae CW63/GWT1 株）。

GWT1 過剰発現株は、前記式（Ｉａ）に記載の化合物に対して耐性を示す以外に、野生株との差異は見られず、他の抗真菌剤シクロヘキシミド、ベノミル、アンホテリシンＢに対して感受性であった。

実施例Ａ１１　GWT1 遺伝子を欠失した S. cerevisiae の作製

S. pombe の his5 遺伝子 (Longtine MS et al, Yeast, 14: 953-961, 1998) を鋳型とし、配列番号２５及び配列番号２６をプライマーとして、両端に GWT1 配列を含む his5 カセットを PCR で増幅した。

S. cerevisiae G2-10 を実施例Ａ１に記載の方法で培養、集菌し、上述の PCR 産物を実施例Ａ１に記載の方法で形質転換した。SD(His⁻)培地で 30℃、5～7日間培養することにより GWT1 欠失株を得た。

GWT1 欠失株は生育が非常に遅いものの、その生育は前記式（Ｉａ）に記載の化合物の影響を受けず、GWT1 遺伝子産物が該化合物の標的であることが示唆された。また、GWT1 欠失株は、高温で生育できない、細胞が膨化しているといった特徴を示し、透過型電子顕微鏡による観察では、電子密度の高い菌体最外層の綿状線維構造が、消失していた。

実施例Ａ１２　GWT1遺伝子産物を過剰発現したS. cerevisiaeにおける前記式（Ｉａ）に記載の化合物の活性

S. cerevisiae CW63株及びGWT1遺伝子を導入したS. cerevisiae CW6

3/GWT1を用い、実施例Ａ2に記載した方法に準じた方法で、前記式（Ｉａ）に記載の化合物の活性を検討した。

その結果、S. cerevisiae CW63株では、培養上清画分中のセファロスポリナーゼ活性が上昇し、細胞壁画分中の活性が低下している前記式（Ｉａ）に記載の化合物濃度（0.39～1.56μg/ml）でも、S. cerevisiae CW63/GWT1株では影響が見られず、またS. cerevisiae CW63株では増殖が抑制される前記式（Ｉａ）に記載の化合物濃度（> 3.13μg/ml）でも、S. cerevisiae CW63/GWT1株では増殖抑制が見られなかった（図６）。

実施例Ａ１３　(4-ブチルフェニル)(1-イソキノリル)ケトンの合成

窒素雰囲気下、マグネシウム338 mg（13.9ミリモル）とテトラヒドロフラン6.5 mlの混合溶液に、１－ブロモ－４－ブチルベンゼン2.29 ml（13.0ミリモル）と開始剤として触媒量の１，２－ジブロモエタンを加え、１０分間還流下撹拌した。この溶液を０℃まで冷却し、１－イソキノリンカルボニトリル1.0 g（6.49ミリモル）のテトラヒドロフラン溶液を加え、さらに室温で１時間、70℃で３時間撹拌した。その後、再度０℃に冷却し、濃塩酸2.56 mlそしてメタノール11 mlを加えた後、２時間加熱還流した。濃縮後残渣を５規定水酸化ナトリウムとトルエンに溶解し、セライトで濾過した。濾液のトルエン層を分配し、水洗、硫酸マグネシウムで乾燥、濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物1.72 gを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.93(3H, t), 1.32-1.43(2H, m), 1.58-1.66(2H, m), 2.68(2H, t), 7.28(2H, d), 7.61(1H, td), 7.74(1H, td), 7.80(1H, d), 7.87(2H, d), 7.92(1H, d), 8.20(1H, d), 8.60(1H, d)

実施例Ａ１４　前記式（Ｉａ）に記載の化合物{1-(4-ブチルベンジル)イソキノリン}の合成

実施例Ａ１３の化合物1.72 g（5.95ミリモル）、ヒドラジン１水和物836 mg（16.7ミリモル）そして水酸化カリウム769 mg（13.7ミリモル）をジエチレングリコール8.5 mlに加え、80℃で１時間、160℃で３時間半そして200℃で１時間攪拌した。室温まで冷却後、氷水を加え酢酸エチルで抽出した。これを水洗後、硫酸マグネシウムで乾燥、濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、前記式（Ｉａ）に記載の化合物を914mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.50-1.59(2H, m), 2.53(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.53(1H, td), 7.56(1H, d), 7.64(1H, td), 7.81(1H, d), 8.18(1H, dd,), 8.50(1H, d)

実施例Ａ１５　前記式（Ｉａ）に記載の化合物{1-(4-ブチルベンジル)イソキノリン}の製造方法の別法

６０％水素化ナトリウム16 mg（0.40ミリモル）のジメチルホルムアミド（1.8 ml）溶液に窒素雰囲気下－１６℃で、Org.Synth.,VI,115(1988)の文献に基づいて合成した1-シアノ-2-ベンゾイル-1,2-ジヒドロイソキノリン100 mg（0.38ミリモル）と４－n-ブチルベンジルクロリド70 mg（0.38ミリモル）のジメチルホルムアミド（3.6 ml）溶液を滴下し、さらに室温で30分間攪拌した。水を加え、濃縮し、残渣にトルエンと水を加えた。トルエン層を水洗後、炭酸カリウムで乾燥後、濃縮した。残渣のエタノール(1.6 ml)溶液に50%水酸化ナトリウム水溶液（0.63 ml）を加え、２時間加熱還流した。濃縮後、トルエンと水を加えた。トルエン層を水洗後、炭酸カルシウムで乾燥後、濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、前記式（Ｉａ）に記載の化合物18 mgを得た。

実施例Ａ１６　S. cereviciae GWT1遺伝子の、C. albicansホモログの

クローニング

HindIII (TaKaRa) で16時間処理した25μgのC. albicansゲノムDNAを、0.75%アガロースゲル電気泳動法により分離し、約3.5から4.5 kbの大きさのDNAフラグメントをゲルから回収した。回収したDNAフラグメントをpKF3ベクター (TaKaRa) のHindIIIサイトに挿入して、カンジダゲノムライブラリを作製した。

作製したライブラリを用いて約1万個のコロニーをLB/Ampicillinプレートにdisplayした後、Colony/Plaque Screen (NEN) メンブレンを用いてコロニーリフトを行いハイブリダイゼーションに供した。プローブは、配列番号1に記載の約1.5 kbのDNAフラグメント20 ngを、ランダムプライマー法によりalpha33P-dCTPでラベルし、GeneQuantカラム (Amersham-Pharmacia) を用いて精製し作製した。

ハイブリダイゼーションは、メンブレンをPerfectHyb™ (TOYOBO) 溶液に浸し65℃で1時間プレインキュベーションをおこなった後、ラベルした上記プローブを添加し、65℃で更に2.5時間インキュベーションした。洗浄は、1).2xSSC, 0.05% SDS溶液：25℃5分、2).2xSSC, 0.05% SDS溶液：25℃15分、3).0.1xSSC, 0.1% SDS溶液50℃20分で行った。洗浄後のメンブレンをサランラップで包み、X-RAY FILM (KONICA) に室温で24時間接触させた後現像した。感光したスポットに相当する大腸菌コロニーを分離して、2次スクリーニングに供した。分離したコロニーをLB/Ampicillinプレートに約200個づつdisplayし、1次スクリーニング同様にコロニーリフトをおこないハイブリダイゼーションに供した。ハイブリダイゼーションの条件は1次スクリーニングと同一の条件でおこなった。

その結果、プローブと強く反応する大腸菌の単一なコロニーが分離された。このコロニーからプラスミドを回収し、含有する配列を決定したところ、実施例A9で見出された配列（配列番号5）と同一の新規配列

が見いだされ（カンジダGWT1の配列）、C. albicansホモログであることが予想された。

実施例Ａ１７　S. cereviciae GWT1遺伝子の、S. Pombeホモログ

データベース検索により、S. cereviciae GWT1遺伝子とホモロジーを示すS. Pombe遺伝子（配列番号２７、及びその遺伝子産物のアミノ酸配列：配列番号２８)が見出され、GWT1のS. Pombeホモログであると考えられた。

実施例Ａ１８　S. cereviciae GWT1遺伝子の、Aspergillus fumigatusホモログのクローニング

発明者らは遺伝子配列解析により、S.cerevisiae, S.pombe, C.albicansのGWT1遺伝子のコードする蛋白において高度に保存されている領域を２カ所見いだした（図７）。この保存領域のアミノ酸をコードするDNAの予測から、配列番号２９、配列番号３０及び配列番号３１のプライマーを設計した。STRATAGENE社から購入したライブラリ（Aspergillus fumigatus cDNA library:#937053) 1μlを鋳型に用いて、配列番号２９および配列番号３１のプライマーを用いてPCR増幅をおこなった。さらにこの増幅サンプル1μlを鋳型に、配列番号２９および配列番号３０のプライマーでnested-PCRをおこなった結果、約250 bpの単一フラグメントの増幅が確認された。このフラグメントの配列を決定したところ配列番号３２に示す、S.cerevisiaeのGWT1遺伝子と相同性を有する新規の配列が得られ、これがA.fumigatusのホモログであることが予想された。

全長のcDNAを獲得するために、増幅フラグメントの配列をもとに配列番号３３および配列番号３４のプライマーを設計した。また、ライブラリの遺伝子挿入部位の外側のプライマー配列番号３５および配列番号３６を設計した。A.fumigatus cDNAライブラリを鋳型にして、配列番号３３および配列番号３５のプライマーセット、または配列番号３４および

配列番号３６のプライマーセットを用いてPCRをおこなった結果、両者から約1 kbのDNAフラグメントの増幅が確認された。これらのフラグメントの塩基配列を決定した結果、配列番号１に示すS.cerevisiaeのGWT1遺伝子と高い相同性を有する新規の配列が得られた。同配列はS.cerevisiae, S.pombe, C.albicansのGWT1遺伝子と全体を通じて高い相同性を有することから、この配列がA.fumigatusのホモログであることが強く示唆された。

A.fumigatusのホモログ全体をクローニングするために、得られた配列をもとに、開始コドン上流に相当する配列番号３７に示すプライマーおよび終止コドン下流に相当するプライマー配列番号３８を新たに設計した。A.fumigatus cDNAライブラリ（STRATAGENE社）およびA.fumigatusゲノムライブラリ（STRATAGENE社）を鋳型に、配列番号３７および配列番号３８のプライマーで３５サイクルのＰＣＲをおこなった結果、両方の鋳型から約1.6kbの単一な増幅フラグメントが検出された。このフラグメントの塩基配列をダイレクトシークエンスによって決定した結果、cDNAライブラリからは配列番号３９に示す塩基配列が見いだされ、配列番号４０に示す501アミノ酸からなる蛋白をコードしていることが示唆された。また、ゲノムライブラリからは配列番号４１に示す塩基配列が見いだされ、77塩基対からなるイントロンを１カ所有していることが判った。

実施例Ａ１９　S. cereviciae GWT1遺伝子の、Cryptococcusホモログのクローニング

1).データベースサーチ

データベースサーチによってS. cereviciae GWT1遺伝子と相同性のある遺伝子を検索した結果、スタンフォード大学のゲノムセンターのサーバー（http://baggage.stanford.edu/cgi-misc/cneoformans/）から、

502042C05.x1の配列を見いだした。また、米国オクラホマ大学のサーバー（http://www.genome.ou.edu/cneo_blast.html）から、b6e06cn.f1の配列を見いだした。

2).ゲノムDNAを鋳型としたPCR

502042C05.x1の配列をもとに配列番号４２のプライマーを作製し、またb6e06cn.f1の配列をもとに配列番号４３のプライマーを作製した。クリプトコッカス（Cryptococcus neoformans）のゲノムDNAを鋳型にして、配列番号４２のプライマーおよび配列番号４３のプライマーを用いてPCR増幅を行ったところ、約2 kbの増幅フラグメントが検出された。このフラグメントの塩基配列を決定したところ、配列番号４４に示す、S. cerevisiaeのGWT1遺伝子と相同性を有する新規の配列が得られた。

クリプトコッカスGWT1遺伝子の開始コドン上流の配列を獲得するために、502042C05.x1の配列をもとに配列番号４５のプライマーを設計し、また配列番号４４の配列をもとに配列番号４６のプライマーを設計した。クリプトコッカスのゲノムDNAを鋳型にして、配列番号４５のプライマーおよび配列番号４６のプライマーを用いてPCR増幅を行ったところ、約500 bpの増幅フラグメントが検出された。このフラグメントの塩基配列を決定したところ、配列番号４７に示す配列が得られ、配列番号４４とオーバーラップすることが判った。

3).3'-RACE

クリプトコッカスGWT1遺伝子の3'末端の配列を得るために、3'-RACEをおこなった。クリプトコッカスから抽出した16μgのtotal RNAをもとに配列番号４８で示すadaptor-primerでプライミングし、SuperScript II Reverse Transcriptase（GIBCO/BRL社製）を用いて逆転写反応をおこない、以降のRT-PCRの鋳型となる１本鎖cDNAを作製した。１本鎖cDNAを鋳型に、配列番号４９および配列番号５０に示すプライマーで35サイ

クルのPCRをおこなった結果、約1.2 kbの増幅フラグメントが検出された。このフラグメントの塩基配列をDirect-Sequence法によって解析したところ、配列番号５１に示す、S.cerevisiaeのGWT1遺伝子と相同性を有する新規の配列が得られた。

4).全長ゲノムDNAのPCR

配列番号４７をもとに設計した配列番号５２のプライマーおよび、配列番号５１をもとに設計した配列番号５３のプライマーを用いて、クリプトコッカスのゲノムDNAを鋳型に独立した3本のpreparationで35サイクルのPCRをおこなった。その結果、独立した3本のtubeからはいずれも約2 kbの増幅フラグメントが検出されたので、それぞれ個別にDirect-Sequenceに供し、全塩基配列を決定した。その結果、3つの独立した配列は完全に一致し、配列番号５４に示すクリプトコッカスのGWT1遺伝子ホモログ全長を含む配列が得られた。

5).cDNA配列の決定

配列番号５４に示すゲノム由来のクリプトコッカスGWT1遺伝子配列を、3'-RACEによって得られたcDNA配列５１と比較することにより、２カ所のイントロンの存在が示唆された。また、開始ATG以降のOpen Reading Frame が通っていないことから、さらにもう１カ所のイントロンの存在が示唆された。そこで、予想されるアミノ酸配列およびスプライシング・ドナー/アクセプター配列から、cDNA構造を予測し、エクソン間のジャンクションと予想される部位に、配列番号５５および配列番号５６で示すプライマーを設計した。クリプトコッカス由来の一本鎖cDNAをテンプレートに上記プライマーを用いて35サイクルのPCRをおこなった結果、約1.4 kbの増幅フラグメントが確認された。同フラグメントをDirect-Sequenceに供し塩基配列の決定をおこなった結果、配列番号５７に示す配列が得られ、配列番号５４と照合することにより、クリプトコッカスのGWT1

遺伝子のcDNA配列が配列番号５８に示す構造であることが示唆された。同配列はS.cerevisiae，S.pombe，C.albicans，A.fumigatusのGWT1遺伝子と部分的に高い相同性を有することから、この配列がクリプトコッカスのホモログであることが強く示唆された。

実施例Ａ２０　前記式（Ｉａ）で表される化合物に対し耐性を付与する遺伝子変異

pRLW63Tを導入することによりリゾチーム遺伝子をレポータ遺伝子として持つ、S. cerevisiae LW63株をメタンスルホン酸エチルで処理した後、前記式（Ｉａ）で表される化合物を1.56，3.13，6.25μg/mlの濃度で含むSD培地で37℃、３日間培養することにより耐性変異株を５株得た(R1～R5)。この内、R1変異株およびR5変異株は、一遺伝子変異により前記式（Ｉａ）で表される化合物に対する特異的な耐性形質を獲得していることがわかった。この２つの突然変異株がGWT1遺伝子上に変異を持っているかどうかを確かめるために、両変異株からゲノムDNAを抽出し、GWT1遺伝子部分について塩基配列決定を行った。この結果、R1変異株では1213番目のグアニンがアデニンに変異していた。またR5変異株では418番目のグアニンからアデニンに変異していた。これによりR1変異株では405番目のアミノ酸であるイソロイシンがバリンに、またR5変異株では140番目のアミノ酸であるグリシンがアルギニンに変わっていることが判明した。

次にこれらの変異が前記式（Ｉａ）で表される化合物に対する特異的な耐性形質獲得の原因となっているかを確かめるために、両変異株由来ゲノムDNAを鋳型として配列番号６０及び６１に記載のプライマーを用いて変異GWT1遺伝子（R1またはR5）を単離した。同時にGWT1のプロモータ領域（配列番号６２）、およびターミネーター領域（配列番号６３）を単離し、GWT1遺伝子プロモータ、変異GWT1遺伝子ORF、およびGWT1遺伝

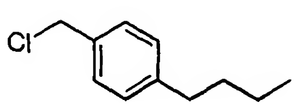
子ターミネーターをpRS316ベクターに挿入して、変異GWT1遺伝子を１コピー発現するプラスミドを構築した(pRS316GWT1-R1, pRS316GWT1-R5)。これをGWT1遺伝子が１コピーのみ破壊されている２倍体株(WDG1)に導入した。このコロニーを胞子形成培地上で培養することにより胞子を形成させ、四分子分析を行うことにより、上記プラスミドを持ち、かつ染色体上のGWT1遺伝子が破壊されているクローンを得た。これを前記式（Ｉａ）で表される化合物を含む培地で培養したところ、もとのR1変異株、R5変異株と同様に、前記式（Ｉａ）で表される化合物に対して耐性を示した。以上のことから、GWT1遺伝子上に起こったアミノ酸変異を伴う点突然変異により前記式（Ｉａ）で表される化合物に対する特異的な耐性形質が付与されることが明らかとなり、この化合物がGWT1タンパク質に直接結合してその機能を阻害していることが強く示唆された。

[実施例Ｂ]

本発明にかかる化合物は、例えば以下の実施例に記載した方法により製造することができる。ただし、これらは例示的なものであって、本発明にかかる化合物は如何なる場合も以下の具体例に制限されるものではない。

実施例Ｂ１

1-（クロロメチル）-4-n-ブチルベンゼン

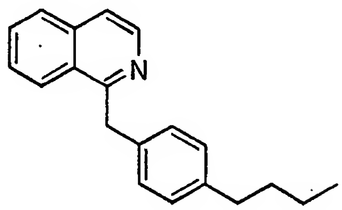


4-n-ブチルベンジルアルコール2.0g（12ミリモル）のエーテル（25ml）溶液に、塩化チオニル2.5ml（34ミリモル）を加え、室温で３時間撹拌した。濃縮後、ベンゼンによる共沸により過剰の塩化チオニルを除去し、表題化合物2.3gを得た。この化合物は精製することなく次の反応に用い

た。

実施例Ｂ２

1-（4-ブチルベンジル）イソキノリン

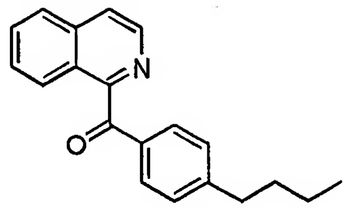


６０％水素化ナトリウム16mg（0.40ミリモル）のジメチルホルムアミド（1.8ml）溶液に窒素雰囲気下－１６℃で、Org. Synth.,VI, 115(1988)の文献に基づいて合成した1-シアノ-2-ベンゾイル-1,2-ジヒドロイソキノリン100mg（0.38ミリモル）と４－n-ブチルベンジルクロリド70mg（0.38ミリモル）のジメチルホルムアミド（3.6ml）溶液を滴下し、さらに室温で３０分間攪拌した。水を加え、減圧濃縮し、残渣にトルエンと水を加えた。トルエン層を水洗後、炭酸カリウムで乾燥後、減圧濃縮した。残渣のエタノール(1.6ml)溶液に５０％水酸化ナトリウム水溶液（0.63ml）を加え、２時間加熱還流した。濃縮後、トルエンと水を加えた。トルエン層を水洗後、炭酸カルシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物18mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.50-1.59(2H, m), 2.53(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.53(1H, td), 7.56(1H, d), 7.64(1H, td), 7.81(1H, d), 8.18(1H, dd), 8.50(1H, d)

実施例Ｂ３

（4-ブチルフェニル）（1-イソキノリル）ケトン



窒素雰囲気下、マグネシウム338mg（14ミリモル）とテトラヒドロフラン6.5mlの混合溶液に、1－ブロモ－4－ブチルベンゼン2.29ml（13ミリモル）と開始剤として触媒量の1，2－ジブロモエタンを加え、10分間還流下撹拌した。この溶液を0℃まで冷却し、1－イソキノリンカルボニトリル1.0g（6.5ミリモル）のテトラヒドロフラン溶液を加え、さらに室温で1時間、70℃で3時間撹拌した。その後、再度0℃に冷却し、濃塩酸2.6mlそしてメタノール11mlを加えた後、2時間加熱還流した。濃縮後、残渣を5規定水酸化ナトリウムとトルエンに溶解し、セライトで濾過した。濾液のトルエン層を分離し、水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物1.7gを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.93(3H, t), 1.32-1.43(2H, m), 1.58-1.66(2H, m), 2.68(2H, t), 7.28(2H, d), 7.61(1H, td), 7.74(1H, td), 7.80(1H, d), 7.87(2H, d), 7.92(1H, d), 8.20(1H, d), 8.60(1H, d)

実施例Ｂ4

1-（4-ブチルベンジル）イソキノリンの製造方法の別法

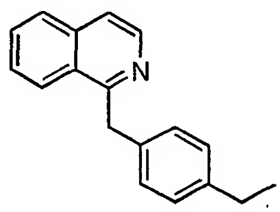
実施例Ｂ3の化合物1.7g（6.0ミリモル）、ヒドラジン1水和物836mg（17ミリモル）そして水酸化カリウム769mg（14ミリモル）をジエチレングリコール8.5mlに加え、80℃で1時間、160℃で3時間半そして200℃で1時間撹拌した。室温まで冷却後、氷水を加え酢酸エチルで抽出した。これを水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮

した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物914mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.50-1.59(2H, m), 2.53(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.53(1H, td), 7.56(1H, d), 7.64(1H, td), 7.81(1H, d), 8.18(1H, dd), 8.50(1H, d)

実施例Ｂ５

1-（4-エチルベンジル）イソキノリン

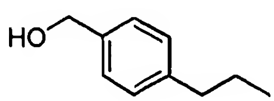


p-エチルベンジルクロリドを用いて実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.18(3H, t), 2.57(2H, q), 4.64(2H, s), 7.08(2H, d), 7.20(2H, d), 7.50-7.55(2H, m), 7.61-7.65(1H, m), 7.80(1H, d), 8.16-8.18(1H, m), 8.49(1H, d)

実施例Ｂ６

（4-プロピルフェニル）メタノール

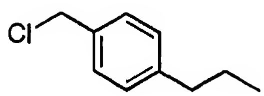


０℃まで冷却した*p*-n-プロピルベゾイックアシッド5.0g(32ミリモル)のテトラヒドロフラン（20ml）溶液に、水素化ホウ素ナトリウム2.9g（76ミリモル）と濃硫酸のエーテル（エーテル4.0mlに濃硫酸2.0mlを加えて調製した。）溶液を反応系内の温度が２０℃以上に上昇しないように滴下し、室温で３時間撹拌した。氷冷後、メタノールと１規定水酸化ナト

リウムを加え酢酸エチルで抽出した。酢酸エチル層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮し、表題化合物を4.33g得た。この化合物は精製することなく次の反応に用いた。

実施例Ｂ７

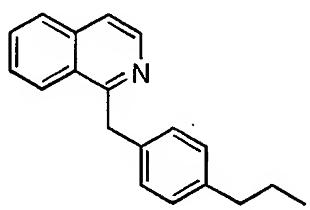
1-（クロロメチル）-4-プロピルベンゼン



実施例Ｂ６の化合物を実施例Ｂ１と同様にして表題化合物を得た。この化合物はさらに精製することなく次の反応に用いた。

実施例Ｂ８

1-（4-プロピルベンジル）イソキノリン

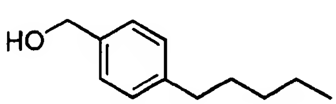


実施例Ｂ７の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.55-1.61(2H, m), 2.51(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.51-7.55(2H, m), 7.61-7.65(1H, m), 7.81(1H, d), 8.17(1H, dd), 8.49(1H, d)

実施例Ｂ９

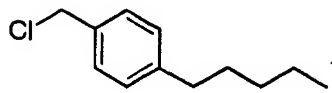
（4-ペンチルフェニル）メタノール



4-n-アミルベンゾイックアシッドを実施例Ｂ６と同様に還元して表題化合物を得た。

実施例Ｂ１０

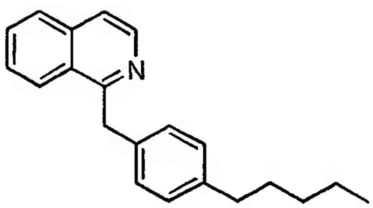
1-（クロロメチル）-4-ペンチルベンゼン



実施例Ｂ９の化合物を実施例Ｂ１と同様にして表題化合物を得た。この化合物はさらに精製することなく次の反応に用いた。

実施例Ｂ１１

1-（4-ペンチルベンジル）イソキノリン

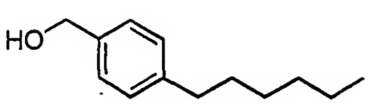


実施例Ｂ１０を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.86(3H, t), 1.26-1.33(4H, m), 1.52-1.59(2H, m), 2.52(2H, t), 4.64(2H, s), 7.06(2H, d), 7.18(2H, d), 7.50-7.55(2H, m), 7.61-7.65(1H, m), 7.80(1H, d), 8.17(1H, dd), 8.49(1H, d)

実施例Ｂ１２

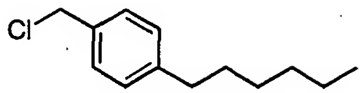
（4-ヘキシルフェニル）メタノール



4-n-ヘキシルベンゾイックアシッドを実施例Ｂ６と同様に還元して表題化合物を得た。この化合物はさらに精製することなく次の反応に用いた。

実施例Ｂ１３

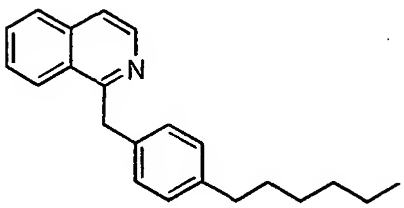
1-（クロロメチル）-4-ヘキシルベンゼン



実施例Ｂ１２の化合物を実施例Ｂ１と同様にして表題化合物を得た。この化合物はさらに精製することなく次の反応に用いた。

実施例Ｂ１４

1-（4-ヘキシルベンジル）イソキノリン

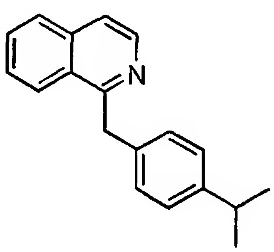


実施例Ｂ１３の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.86(3H, t), 1.26-1.31(6H, m), 1.51-1.58(2H, m), 2.52(2H, t), 4.63(2H, s), 7.06(2H, d), 7.18(2H, d), 7.50-7.55(2H, m), 7.61-7.65(1H, m), 7.80(1H, d), 8.17(1H, dd), 8.49(1H, d)

実施例Ｂ１５

1-（4-イソプロピルベンジル）イソキノリン

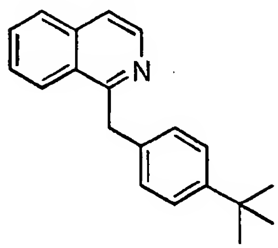


p-イソプロピルベンジルクロリドを実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):1.19(6H, d), 2.80-2.87(1H, m), 4.64(2H, s), 7.11(2H, d), 7.21(2H, d), 7.51-7.56(2H, m), 7.61-7.65(1H, m), 7.81(1H, d), 8.19(1H, dd), 8.50(1H, d)

実施例Ｂ１６

1-[4-(tert-ブチル)ベンジル]イソキノリン

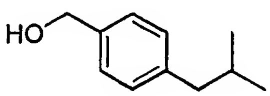


4-tert-ブチルベンジルクロリドを実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.26(9H, s), 4.64(2H, s), 7.22(2H, d), 7.27(2H, d), 7.52-7.56(2H, m), 7.62-7.66(1H, m), 7.81(1H, d), 8.19(1H, dd), 8.50(1H, d)

実施例Ｂ１７

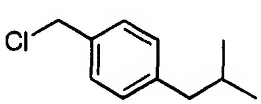
(4-イソブチルフェニル)メタノール



4-イソブチルベンゾイックアシッドを実施例Ｂ６と同様に還元して表題の化合物を得た。さらに精製することなく次の反応に用いた。

実施例Ｂ１８

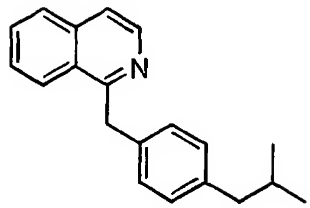
1-(クロロメチル)-4-イソブチルベンゼン



実施例Ｂ１７の化合物を実施例Ｂ１と同様にして表題化合物を得た。さらに精製することなく次の反応に用いた。

実施例Ｂ１９

1-(4-イソブチルベンジル)イソキノリン

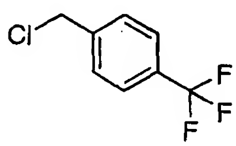


実施例B18の化合物を実施例B2と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.86(6H, d), 1.75-1.83(1H, m), 2.39(2H, d), 4.66(2H, s), 7.02(2H, d), 7.18(2H, d), 7.52-7.58(2H, m), 7.63-7.67(1H, m), 7.82(1H, d), 8.18(1H, d), 8.50(1H, d)

実施例B20

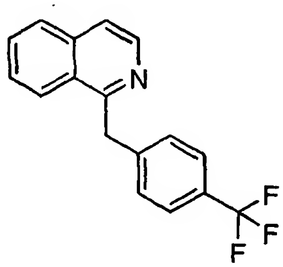
1-(クロロメチル)-4-(トリフルオロメチル)ベンゼン



4-トリフルオロメチルベンジルアルコールを実施例B1と同様にして表題化合物を得た。さらに精製することなく次の反応に用いた。

実施例B21

1-[4-(トリフルオロメチル)ベンジル]イソキノリン

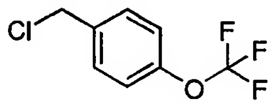


実施例B20の化合物を実施例B2と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.73(2H, s), 7.39(2H, d), 7.51(2H, d), 7.54-7.60(2H, m), 7.65-7.69(1H, m), 7.84(1H, d), 8.09-8.10(1H, m), 8.51(1H, d)

実施例B22

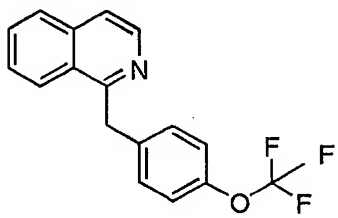
1-(クロロメチル)-4-(トリフルオロメトキシ)ベンゼン



4-トリフルオロメトキシベンジルアルコールを実施例Ｂ１と同様にして表題化合物を得た。さらに精製することなく次の反応に用いた。

実施例Ｂ２３

1-[4-(トリフルオロメトキシ)ベンジル]イソキノリン

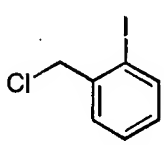


実施例Ｂ２２の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.67(2H, s), 7.10(2H, d), 7.27(2H, d), 7.54-7.59(2H, m), 7.64-7.68(1H, m), 7.84(1H, d), 8.11(1H, dd), 8.50(1H, d)

実施例Ｂ２４

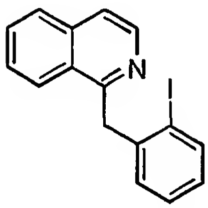
1-(クロロメチル)-2-ヨードベンゼン



０℃に冷却したo-ヨードベンジルアルコール5.0g（21ミリモル）の塩化メチレン（50ml）溶液に、メタンスルフォニルクロリド2.0ml（29ミリモル）とトリエチルアミン3.6ml（26ミリモル）を加え、その温度で１９時間撹拌した。５％炭酸水素ナトリウム水溶液を加え、塩化メチレンで抽出した。塩化メチレン層を無水硫酸マグネシウムで乾燥後、減圧濃縮し、表題化合物を5.34g 得た。

実施例Ｂ２５

1-(2-ヨードベンジル)イソキノリン

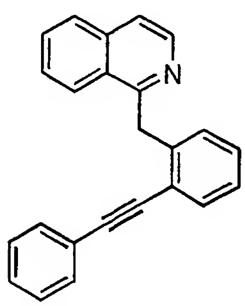


実施例Ｂ２４の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):4.74(2H, s), 6.81-6.84(1H, m), 6.87-6.92(1H, m), 7.11-7.15(1H, m), 7.55-7.57(1H, m), 7.60(1H, d), 7.64-7.68(1H, m), 7.83-7.86(1H, m), 7.89-7.91(1H, m), 8.00-8.02(1H, m), 8.50(1H, d)

実施例Ｂ２６

1-[2-(2-フェニル-1-エチニル)ベンジル]イソキノリン



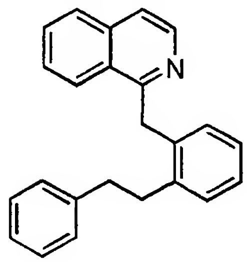
窒素雰囲気下、実施例Ｂ２５の化合物345mg（1.07ミリモル）のピロリジン（1.5ml）溶液に、テトラキストリフェニルフォスフィンパラジウム58mg（0.05ミリモル）とエチニルベンゼン204mg（2.0ミリモル）のピロリジン（1.5ml）溶液を加え、８０℃で３時間攪拌した。室温まで冷却後、酢酸エチルで希釈後、飽和塩化アンモニウム水溶液で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルクロマトグラフィーで精製し、表題化合物280mgを得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):4.95(2H, s), 6.98-7.06(2H, m), 7.10-7.21

(2H, m), 7.31-7.35(3H, m), 7.48-7.51(3H, m), 7.57-7.65(2H, m), 7.82(1H, d), 8.25(1H, d), 8.52(1H, d)

実施例Ｂ２７

1-(2-フェニルエチルベンジル)イソキノリン

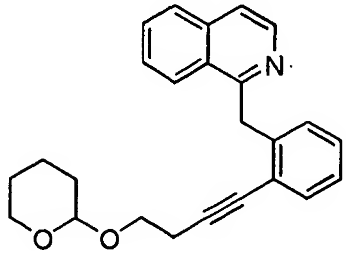


実施例Ｂ２６の化合物280mg(0.88ミリモル)のテトラヒドロフラン(30ml)溶液に、パラジウム－炭素（10%）230mgを加え、室温で水素雰囲気下（1 atm）で３時間撹拌した。触媒を濾去し、得られた濾液を減圧濃縮した。残渣をシリカゲルクロマトグラフィーで精製し、表題化合物162mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.90-2.94(2H, m), 3.07-3.10(2H, m), 4.67(2H, s), 6.80(1H, d), 7.02-7.06(1H, m), 7.15-7.30(7H, m), 7.49-7.53(1H, m), 7.58(1H, d), 7.64-7.68(1H, m), 7.84(1H, d), 7.95(1H, d), 8.50(1H, d)

実施例Ｂ２８

1-{2-[4-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ブチニル]ベンジル}イソキノリン



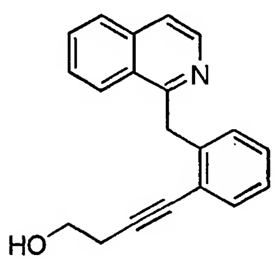
窒素雰囲気下、実施例Ｂ２５の化合物345mg（1.07ミリモル）のピロリジン（1.5ml）溶液に、テトラキストリフェニルフォスフィンパラジウム

58mg（0.05ミリモル）と2-(3-ブチニルオキシ)-テトラヒドロ-2H-ピラン208mg（2.0ミリモル）のピロリジン（1.5ml）溶液を加え、４日間室温で撹拌し、さらに８０℃で３０分間撹拌した。室温まで冷却後、酢酸エチルで希釈後、飽和塩化アンモニウム水溶液で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルクロマトグラフィーで精製し、表題化合物277mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.42-1.60(4H, m), 1.64-1.68(1H, m), 1.75-1.81(1H, m), 2.76-2.80(2H, m), 3.46-3.51(1H, m), 3.60-3.66(1H, m), 3.85-3.95(2H, m), 4.64-4.66(1H, m), 4.85(2H, s), 6.95-6.98(1H, m), 7.05-7.13(2H, m), 7.44-7.46(1H, m), 7.49-7.53(1H, m), 7.56(1H, d), 7.60-7.65(1H, m), 7.80-7.82(1H, m), 8.15-8.18(1H, m), 8.49-8.51(1H, m)

実施例Ｂ２９

4-[2-(1-イソキノリルメチル)フェニル]-3-ブチン-1-オール



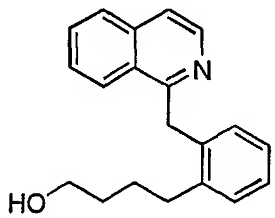
実施例Ｂ２８の化合物200mg（0.54ミリモル）を０℃まで冷却した後、塩酸ーメタノール溶液（10%）を5ml加え、１５分間撹拌した。飽和炭酸水素ナトリウム水溶液を加え、酢酸エチルで抽出した。酢酸エチル層を無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルクロマトグラフィーで精製し、表題化合物86mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.72(2H, t), 3.53-3.60(1H, brs), 3.85(2H, t), 4.85(2H, s), 7.12-7.15(2H, m), 7.22-7.24(1H, m), 7.42-7.44(1H, m), 7.55-7.59(2H, m), 7.63-7.67(1H, m), 7.81(1H, d), 8.30

(1H, m), 8.46(1H, m)

実施例B30

4-[2-(1-イソキノリルメチル)フェニル]-1-ブタノール

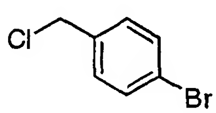


実施例B29の化合物44mg(0.15ミリモル)のテトラヒドロフラン(5ml)溶液に、パラジウム－炭素(10%)10mgを加え、室温で水素雰囲気下(1 atm)1時間撹拌した。触媒を濾去後、減圧濃縮した。残渣をシリカゲルクロマトグラフィーで精製し、表題化合物18mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.61-1.75(4H, m), 2.33(1H, brs), 2.77(2H, t), 3.67(2H, t), 4.70(2H, s), 6.91(1H, d), 7.02-7.06(1H, m), 7.12-7.16(1H, m), 7.19-7.21(1H, m), 7.50-7.55(1H, m), 7.57(1H, d), 7.63-7.67(1H, d), 7.83(1H, d), 8.09(1H, d), 8.47(1H, d)

実施例B31

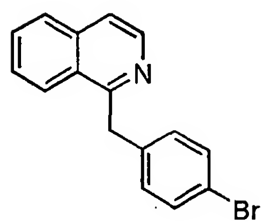
1-ブロモ-2-(クロロメチル)ベンゼン



p-ブロモベンジルアルコールを実施例B1と同様にして表題化合物を得た。

実施例B32

1-(4-ブロモベンジル)イソキノリン

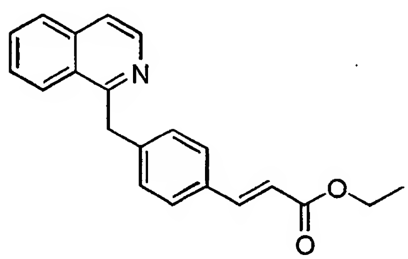


実施例Ｂ３１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.61(2H, s), 7.14-7.16(2H, m), 7.35-7.39 (2H, m), 7.52-7.58(2H, m), 7.63-7.67(1H, m), 7.82(1H, d), 8.07-8.10(1H, m), 8.49(1H, d)

実施例Ｂ３３

エチル(E)-3-[4-(イソキノリルメチル)フェニル]-2-プロペノエート

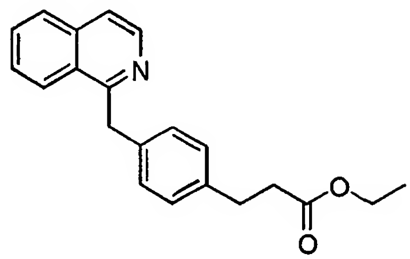


窒素雰囲気下、実施例Ｂ３２の化合物100mg（0.34ミリモル）とプロピオン酸ビニルエステル73μl（0.67ミリモル）のジメチルホルムアミド1.0ml溶液に、トリス（2-メチルフェニル）ホスフィン20mg（0.067ミリモル）、パラジウム(II)アセテート7.5mg（0.034ミリモル）そしてトリエチルアミン70μl（0.50ミリモル）を加え、４時間１００℃で加熱撹拌した。この溶液を室温まで戻した後、水を加え、酢酸エチルで抽出した。有機層を水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物74mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.32(3H, t), 4.24(2H, q), 4.69(2H, s), 6.36(1H, d), 7.29(2H, d), 7.42(2H, d), 7.53-7.67(4H, m), 7.83(1H, d), 8.11-8.13(1H, m), 8.50(1H, d)

実施例B34

エチル3-[4-(1-イソキノリルメチル)フェニル]プロパノエート

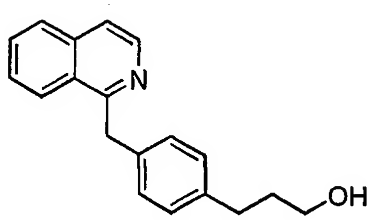


実施例B33の化合物71mg(0.22ミリモル)のメタノール(5.0ml)溶液に、パラジウム-炭素(10%、20mg)を加え、室温で常圧水素雰囲気下、5時間半撹拌した。反応液より触媒を濾別した後、濾液を減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物52mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.20(3H, t), 2.56(2H, t), 2.88(2H, t), 4.09(2H, q), 4.64(2H, s), 7.09(2H, d), 7.20(2H, d), 7.51-7.57(2H, m), 7.62-7.66(1H, m), 7.82(1H, d), 8.15(1H, dd), 8.50(1H, d)

実施例B35

3-[4-(1-イソキノリルメチル)フェニル]-1-プロパノール



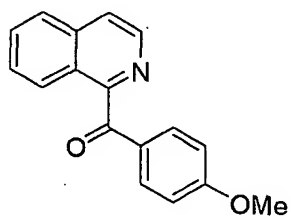
窒素雰囲気下、0℃に冷却したテトラヒドロフラン1.0mlにリチウムアルミニウムヒドリド6mg(0.16ミリモル)を加えた。この溶液に実施例B34の化合物46mg(0.14ミリモル)のテトラヒドロフラン(1.0ml)溶液を加え、その温度で3時間撹拌した。反応液にメタノールと水(9:1, 1.0ml)の混合液を加え、さらに飽和塩化アンモニウム水溶液を加えた後、クロロホルムで抽出した。有機層を無水硫酸マグネシウムで乾燥後、減

圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物22mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.30-1.35(1H, brs), 1.81-1.88(2H, m), 2.64(2H, t), 3.62-3.65(2H, m), 4.64(2H, s), 7.09(2H, d), 7.20(2H, d), 7.51-7.57(2H, m), 7.62-7.66(1H, m), 7.81(1H, d), 8.16-8.18(1H, m), 8.49(1H, d)

実施例Ｂ３６

1-イソキノリル(4-メトキシフェニル)ケトン

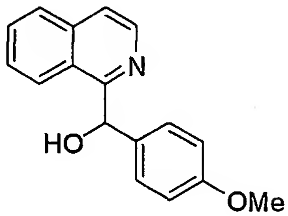


窒素雰囲気下、マグネシウム3059mg(125.8ミリモル)とテトラヒドロフラン(20ml)の混合溶液に、4-ブロモアニソール15.3ml (122ミリモル)と開始剤として触媒量の１，２－ジブロモエタンを加え、加熱還流下45分間撹拌した。この溶液を０℃まで冷却し、1-イソキノリンカルボニトリル10.78g(69.9ミリモル)のテトラヒドロフラン溶液(30ml)を滴下後、室温で2時間撹拌した。反応混合物を氷冷し、濃塩酸24mlとメタノール120mlを加え、1.5時間加熱還流した。氷冷後、水酸化ナトリウム水溶液を加えpH8とした後、エーテルで抽出し、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物15.87gを得た。

^{1}H-NMR($CDCl_3$) δ(ppm):3.88(3H, s), 6.95(2H, d), 7.61(1H, dd), 7.74(1H, dd), 7.76(1H, d), 7.85(2H, d), 8.17(1H, dd), 8.60(1H, d).

実施例Ｂ３７

1-イソキノリル(4-メトキシフェニル)メタノール



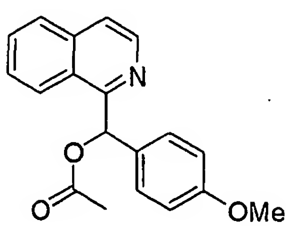
氷冷した実施例Ｂ３６の化合物8608mgのエタノール(170ml)溶液に、水素化ホウ素ナトリウム1855mgを加え、室温で35分間攪拌した。さらに水素化ホウ素ナトリウム957mgを加え40℃で40分間攪拌した。反応混合物を減圧濃縮し、水を加えエーテルで抽出した。有機層を水と飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。得られた表題化合物7881mg はさらに精製することなく次反応に用いた。

^{1}H-NMR(DMSO-d6)δ(ppm):3.66(3H, s), 6.30-6.32(1H, brs), 6.81(2H, d), 7.28(2H, d), 7.54(1H, dd), 7.68(1H, dd), 7.76(1H, d), 7.94(1H, d), 8.37(1H, d), 8.47(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ３８

1-イソキノリル(4-メトキシフェニル)メチルアセテート



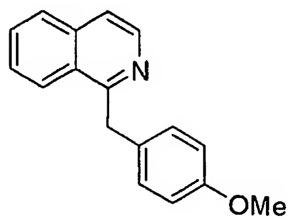
実施例Ｂ３７の化合物7881mgのピリジン(100ml)溶液に、無水酢酸20mlを加え、50℃で4時間攪拌した。反応混合物を減圧濃縮後、さらにトルエン共沸した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物8.79g を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.22(3H, s), 3.76(3H, s), 6.84(2H, d), 7.

39(2H, d), 7.54(1H, dd), 7.56(1H, s), 7.60(1H, d), 7.64(1H, dd), 7,82(1H, d), 8.19(1H, d), 8.57(1H, d).

実施例Ｂ３９

1-(4-メトキシベンジル)イソキノリン

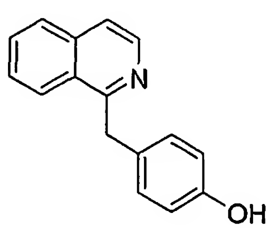


実施例Ｂ３８の化合物8.79gのメタノール(150ml)溶液に、10%パラジウム-炭素4.0gを加え、室温で常圧水素雰囲気下5.5時間撹拌した。触媒をセライトで濾去し、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物4.48gを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.74(3H, s), 4.61(2H, s), 6.79(2H, d), 7.21(2H, d), 7.53(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.16(1H, d), 8.49(1H, d).

実施例Ｂ４０

4-(1-イソキノリルメチル)フェノール

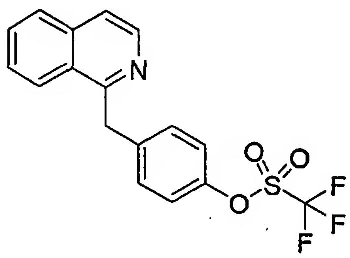


実施例Ｂ３９の化合物2185mgに47%臭化水素酸水溶液40mlを加え、14時間加熱還流した。室温まで戻した後、さらに氷冷し50%水酸化ナトリウム水溶液で中和し、酢酸エチルで抽出した。酢酸エチル層を水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。得られた粉末を石油エーテルで洗浄し、表題化合物1822mgを得た。

^{1}H-NMR(DMSO-d6)δ(ppm):4.48(2H, s), 6.61(2H, d), 7.07(2H, d), 7.60(1H, dd), 7.68(1H, d), 7.71(1H, dd), 7.92(1H, d), 8.27(1H, d), 8.41(1H, d), 9.19(1H, brs).

実施例Ｂ４１

4-(1-イソキノリルメチル)フェニルトリフルオロメタンスルホネート

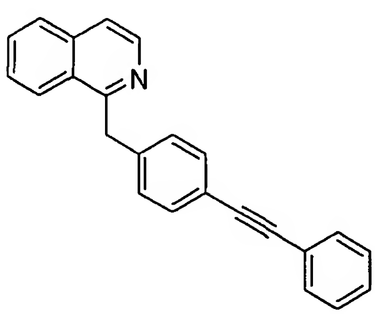


氷冷した実施例Ｂ４０の化合物513mgのピリジン(10ml)溶液に、トリフルオロメタンスルフォン酸無水物0.55mlを滴下し、その温度で45分間攪拌した。その反応溶液に氷を加えエーテルで抽出した。有機層を水と飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物を546mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.69(2H, s), 7.16(2H, d), 7.35(2H, d), 7.57(1H, dd), 7,60(1H, d), 7.68(1H, dd), 7.85(1H, d), 8.09(1H, d), 8.50(1H, d).

実施例Ｂ４２

1-[4-(2-フェニル-1-エチニル)ベンジル]イソキノリン



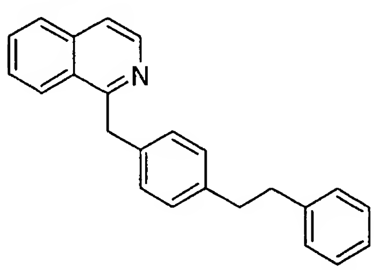
脱気した後、窒素置換した実施例Ｂ４１の化合物88mgの*N,N*-ジメチル

ホルムアミド(2.0ml)溶液に、フェニルアセチレン53μl、酢酸パラジウム9mg、1,1'-ビス(ジフェニルフォスフィノ)フェロセン67mg、ヨウ化銅(I)3mg、塩化リチウム20mgそしてトリエチルアミン50μlを加え、80℃で8時間撹拌した。室温まで戻した後、水を加え酢酸エチルで抽出した。有機層を水と飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物53mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.69(2H, s), 7.12-7.32(3H, m), 7.25(2H, d), 7.42(2H, d),7.43-7.52(2H, m), 7.54(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.10(1H, d), 8.51(1H, d).

実施例B43

1-(4-フェネチルベンジル)イソキノリン

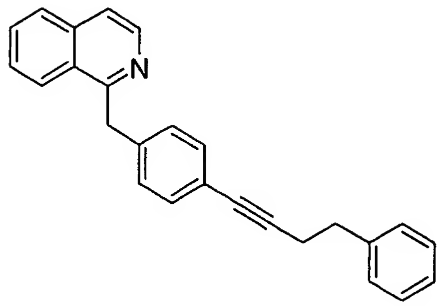


実施例B42の化合物45mgのテトラヒドロフラン(2ml)溶液に、10%パラジウム-炭素触媒20mgを加え、室温で常圧水素雰囲気下2時間撹拌した。触媒をセライトで濾去し、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物23mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.78-2.90(4H, m), 4.64(2H, s), 7.07(2H, d), 7.10-7.20(5H, m), 7.22(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.15(1H, d), 8.49(1H, d).

実施例B44

1-[4-(4-フェニル-1-ブチニル)ベンジル]イソキノリン

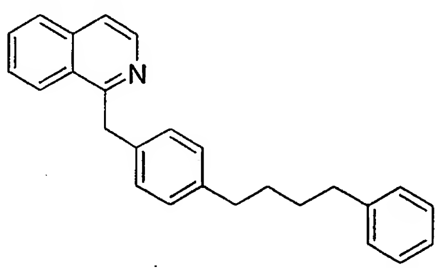


実施例Ｂ４１の化合物と4-フェニル-1-ブチンを用い、実施例Ｂ４２と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.65(2H, t), 2.88(2H, t), 4.68(2H, s), 7.12-7.40(9H, m), 7.50-7.70(3H, m), 7.80-7.88(1H, m), 8.00-8.10(1H, m), 8.48-8.51(1H, m).

実施例Ｂ４５

1-[4-(4-フェニル-1-ブチル)ベンジル]イソキノリン

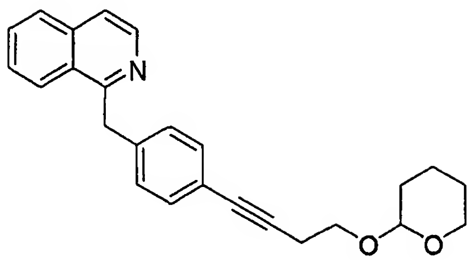


実施例Ｂ４４の化合物を実施例Ｂ４３と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.55-1.80(4H, m), 2.50-2.65(4H, m), 4.68(2H, s), 7.00-7.30(9H, m), 7.52(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.81(1H, d), 8.15(1H, d), 8.50(1H, d).

実施例Ｂ４６

1-{4-[4-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ブチニル]ベンジル}イソキノリン

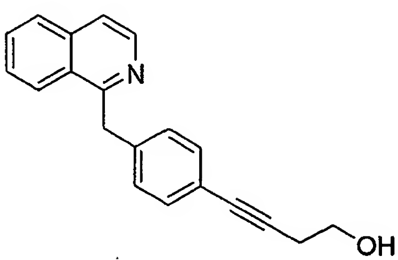


実施例Ｂ４１の化合物と2-(3-ブチニルオキシ)テトラヒドロ-2H-ピランを用い、実施例Ｂ４２と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.48-1.90(6H, m), 2.67(2H, t), 3.49-3.55(1H, m), 3.60(1H, dd), 3.65-3.94(2H, m), 4.66(2H, s), 4.65-4.70(1H, m), 7.14-7.20(2H, m), 7.23-7.30(2H, m), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.10(1H, d), 8.49(1H, d).

実施例Ｂ４７

4-[4-(1-イソキノリルメチル)フェニル]-3-ブチン-1-オール



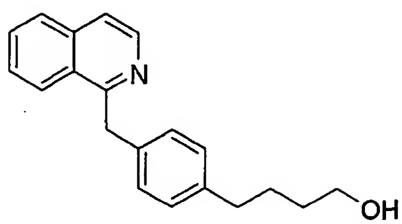
実施例Ｂ４６の化合物1048mgを10%塩酸-メタノール溶液50mlに溶解し、室温で1.5時間攪拌した。反応混合物を氷冷し、飽和炭酸水素ナトリウム水溶液を加え酢酸エチルで抽出した。有機層を水、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物666mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.65(2H, t), 3.77(2H, t), 4.65(2H, s), 7.18(2H, d), 7.29(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.07(1H, d), 8.49(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ４８

4-[4-(1-イソキノリルメチル)フェニル]-1-ブタノール



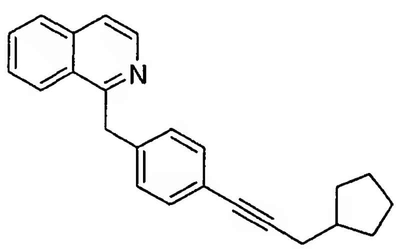
実施例Ｂ４７の化合物を実施例Ｂ４３と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.50-1.70(4H, m), 2.57(2H, t), 3.62(2H, t), 4.64(2H, s), 7.06(2H, d), 7.18(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.16(1H, d), 8.49(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ４９

1-[4-(3-シクロペンチル-1-プロピニル)ベンジル]イソキノリン

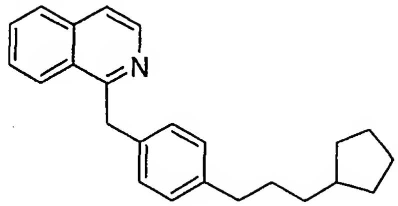


実施例Ｂ４１と3-シクロペンチル-1-プロピンを用い、実施例Ｂ４２と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.25-1.35(2H, m), 1.45-1.70(6H, m), 1.75-1.85(2H, m), 2.05-2.13(1H, m), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.51(1H, dd), 7.56(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.49(1H, d).

実施例Ｂ５０

1-[4-(3-シクロペンチルプロピル)ベンジル]イソキノリン

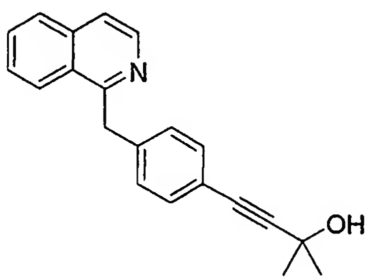


実施例Ｂ４９を実施例Ｂ４３と同様に処理して表題化合物を得た。

[1]H-NMR($CDCl_3$)δ(ppm):1.25-1.74(13H, m), 2.49-2.54(2H, m), 4.64(2H, s), 7.06(2H, d), 7.18(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.17(1H, d), 8.49(1H, d).

実施例Ｂ５１

4-[4-(1-イソキノリルメチル)フェニル]-2-メチル-3-ブチン-2-オール

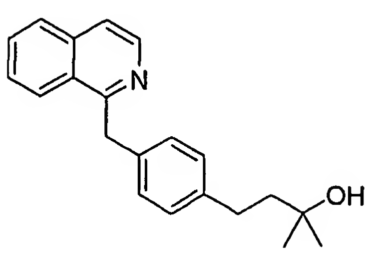


実施例Ｂ４１の化合物と2-メチル-3-ブチン-2-オールを用いて実施例Ｂ４２と同様に処理して表題化合物を得た。

[1]H-NMR(DMSO-d6)δ(ppm):1.35(1H, s), 1.40(6H, s), 4.62(2H, s), 7.20~7.30(4H, m), 7.61(1H, dd), 7.71(1H, d), 7.69-7.76(1H, m), 7.95(1H, d), 8.26(1H, d), 8.42(1H, d).

実施例Ｂ５２

4-[4-(1-イソキノリルメチル)フェニル]-2-メチル-2-ブタノール



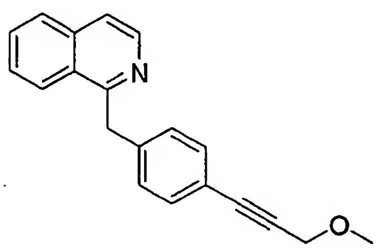
実施例Ｂ５１の化合物を実施例Ｂ４３と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.25(6H, s), 1.70-1.77(2H, m), 2.60-2.67(2H, m), 4.64(2H, s), 7.08(2H, d), 7.19(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.16(1H, d), 8.49(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ５３

1-[4-(3-メトキシ-1-プロピニル)ベンジル]イソキノリン

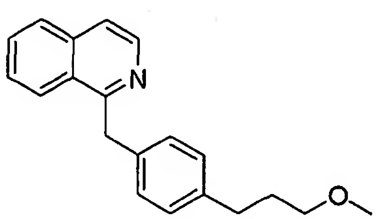


実施例Ｂ４１の化合物とメチルプロパルギルエーテルを用いて、実施例Ｂ４２と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.42(3H, s), 4.29(2H, s), 4.66(2H, s), 7.21 (2H, d), 7.34(2H, d), 7.54(1H, dd), 7.58(1H, d), 7.65(1H, dd) 7.82(1H, d), 8.10(1H, d) 8.49(1H, d).

実施例Ｂ５４

1-[4-(3-メトキシプロピル)ベンジル]イソキノリン

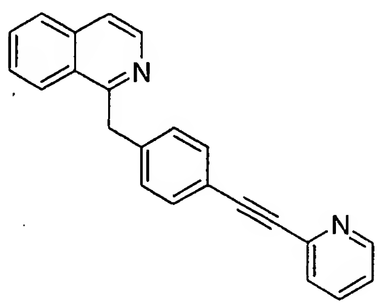


実施例Ｂ５３の化合物を実施例Ｂ４３と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.78-1.87 (2H, m), 2.06(2H, t), 3.31(3H, s), 3.35(2H, t), 4.64(2H, s), 7.07(2H, d), 7.22(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.17(1H, d),8.49(1H, d).

実施例Ｂ５５

1-{4-[2-(2-ピリジル)-1-エチニル]ベンジル}イソキノリン

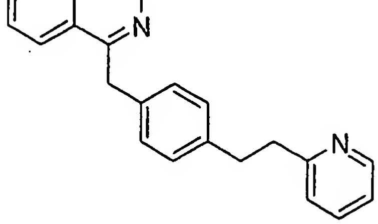


実施例Ｂ４１の化合物と2-エチニルピリジンを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.71(2H, s), 7.20-7.25(2H, m), 7.29(2H, d), 7.48-7.53(1H, m), 7.51(2H, d), 7.57(1H, dd), 7.61(1H, d), 7.67(1H, dd), 7.85(1H, d), 8.13(1H, d), 8.53(1H, d), 8.59-8.63(1H, m).

実施例Ｂ５６

1-{4-[2-(2-ピリジル)エチル]ベンジル}イソキノリン



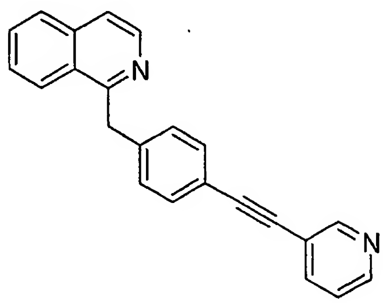
実施例Ｂ５５の化合物を実施例Ｂ４３と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.94-3.06(4H, m), 4.64(2H, s), 7.04(1H,

d), 7.09(1H, dd), 7.09(2H, d), 7.18(2H, d), 7.53(1H, ddd), 7.54(1H, dd), 7.55(1H, d), 7.64(1H, d), 7.81(1H, d), 8.15(1H, d), 8.49(1H, d), 8.53(1H, dd).

実施例Ｂ５７

1-{4-[2-(3-ピリジル)-1-エチニル]ベンジル}イソキノリン

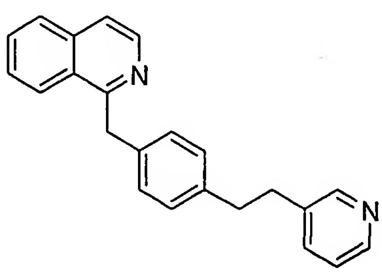


実施例Ｂ４１の化合物と3-エチニルピリジンを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.69(2H, s), 7.27(2H, d), 7.31(1H, dd), 7.43(2H, d), 7.55(1H, dd), 7.59(1H, d), 7.66(1H, dd), 7.82(1H, ddd), 7.83(1H, d), 8.10(1H, d), 8.51(1H, d), 8.60(1H, dd), 8.77(1H, d).

実施例Ｂ５８

1-{4-[2-(3-ピリジル)エチル]ベンジル}イソキノリン



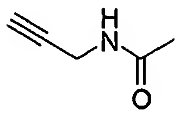
実施例Ｂ５７を実施例Ｂ４３と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.80-2.90(4H, m), 4.65(2H, s),7.04(2H, d), 7.15(1H, dd), 7.19(2H, d), 7.39(1H, dd), 7.54(1H, dd), 7.56(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.15(1H, d), 8.40(1H, d), 8.

42(1H, d), 8.49(1H, d).

実施例Ｂ５９

N-(2-プロピニル)アセトアミド

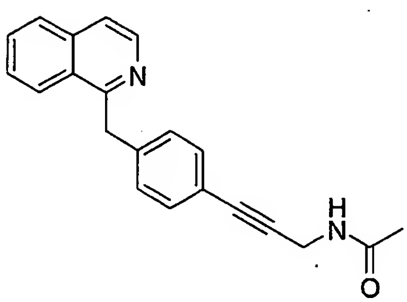


氷冷したプロパルギルアミン3023mgの塩化メチレン(30ml)溶液に、ピリジン16.3mlと無水酢酸10.4mlを加え、室温で1時間撹拌した。反応混合物を氷に注ぎ、酢酸エチルで抽出し、1規定塩酸、飽和炭酸水素ナトリウム水溶液そして飽和食塩水で洗浄後、無水硫酸マグネシウムで乾燥後、シリカゲルを用いて濾過した。減圧濃縮し、表題化合物743mg を得た。得られた化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR(DMSO-d6)δ(ppm):1.79(3H, s), 3.07(1H, t), 3.81(2H, d), 8.25(1H, brs).

実施例Ｂ６０

N-{3-[4-(1-イソキノリルメチル)フェニル]-2-プロピニル}アセトアミド

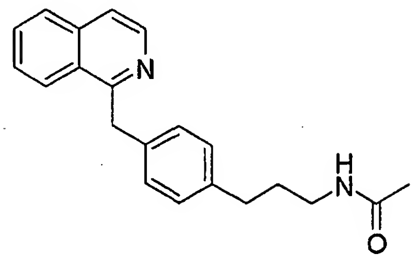


実施例Ｂ４１の化合物と実施例Ｂ５９の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR(DMSO-d6)δ(ppm):1.79(3H, s), 4.04(2H, s), 4.61(2H, s), 7.45-7.68(4H, m), 7.68-7.75(2H, m), 7.90-8.00(1H, m), 8.25-8.38(2H, m), 8.40-8.45(1H, m).

実施例Ｂ６１

N-{3-[4-(1-イソキノリルメチル)フェニル]プロピル}アセトアミド

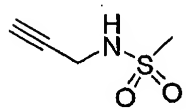


実施例Ｂ６０を実施例Ｂ４３と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.95(3H, s), 1.74-1.84(2H, m), 2.55(2H, t), 3.25(2H, dt), 4.68(2H, s), 7.10(2H, d), 7.18(2H, d), 7.20-7.28(1H, m), 7.50-7.58(2H, m), 7.60-7.68(1H, m), 7.75-7.85(1H, m), 8.10-8.16(1H, m), 8.45-8.50(1H, m).

実施例Ｂ６２

N-(2-プロピニル)メタンスルホンアミド



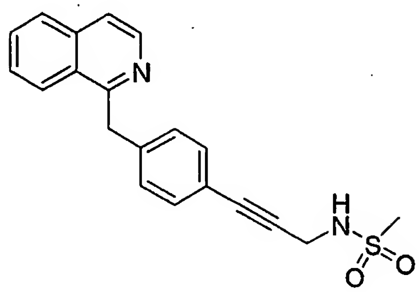
氷冷したプロパルギルアミン3023mgの塩化メチレン(30ml)溶液に、トリエチルアミン9.77mlを加え、メタンスルホニルクロリド5.19mlを滴下した後、その温度で3時間攪拌し、その後室温に昇温し、さらに2時間攪拌した。反応混合物に氷を加え、酢酸エチルで抽出し、飽和食塩水で洗浄後、無水硫酸マグネシウムで乾燥し、減圧濃縮した。残渣をメタノール120mlに溶解し、炭酸カリウム11.7gを加え、室温で3時間攪拌した。反応混合物を減圧濃縮し、氷冷下希塩酸で中和した後、酢酸エチルで抽出した。飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物6.67gを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.39(1H, t), 3.10(3H, s), 3.99(2H, dd), 4.

60(1H, brs).

実施例Ｂ６３

N-{3-[4-(1-イソキノリルメチル)フェニル]-2-プロピニル}メタンスルホンアミド

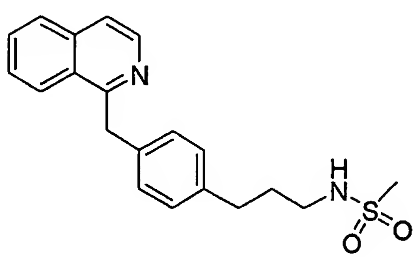


実施例Ｂ４１の化合物と実施例Ｂ６２の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR(DMSO-d6)δ(ppm):2.97(3H, s), 4.00(2H, d), 4.63(2H, s), 7.25-7.37(4H, m), 7.57(1H, t), 7.62(1H, dd), 7.71(1H, d), 7.73(1H, dd), 7.94(1H, d), 8.28(1H, d), 8.42(1H, d).

実施例Ｂ６４

N-{3-[4-(1-イソキノリルメチル)フェニル]プロピル}メタンスルホンアミド



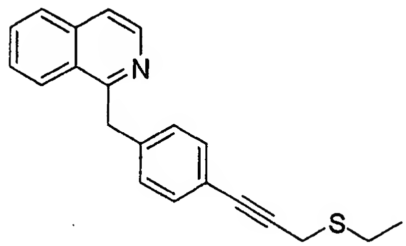
実施例Ｂ６３の化合物を実施例Ｂ４３と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.80-1.90(2H, m), 2.62(2H, t), 2.89(3H, s), 3.11(2H, dt), 4.25(1H, brs), 4.64(2H, s), 7.05(2H, d), 7.20(2H, d), 7.50(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.81(1H, d),

8.15(1H, d), 8.49(1H, d).

実施例Ｂ６５

1-{4-[3-(エチルスルファニル)-1-プロピニル]ベンジル}イソキノリン

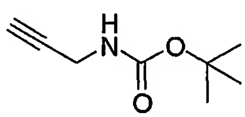


実施例Ｂ４１の化合物とプロパルギルエチルスルフィドを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.30(3H, t), 2.73(2H, q), 3.47(2H, s), 4.67(2H, s), 7.20-7.32(4H, m), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.49(1H, d).

実施例Ｂ６６

t-ブチル*N*-(2-プロピニル)カルバメート



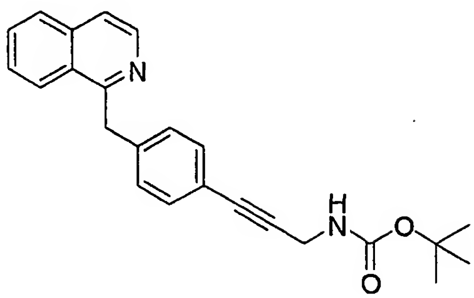
氷冷したプロパルギルアミン3040mgのテトラヒドロフラン(20ml)溶液に、ジ-t-ブチル-ジカルボナート10.84gのテトラヒドロフラン溶液（20ml）を滴下し、徐々に室温まで昇温し、20時間撹拌した。反応混合物に水を加え、酢酸エチルで抽出し、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮し、表題化合物9.34gを得た。得られた化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR(DMSO-d6)δ(ppm):1.36(9H, s),3.04(1H, t),3.62-3.70(2H, m),7.20-7.30(1H, m)

実施例Ｂ６７

tert-ブチル　*N*-{3-[4-(1-イソキノリルメチル)フェニル]-2-プロピニ

ル}カルバメート

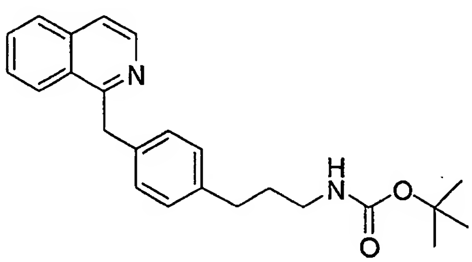


実施例Ｂ４１の化合物と実施例Ｂ６６の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

[1]H-NMR($CDCl_3$)δ(ppm):1.45(9H, s), 4.06-4.13(2H, m), 4.66(2H, s), 7.19(2H, d), 7.20-7.28(1H, m), 7.29(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.08(1H, d), 8.49(1H, d).

実施例Ｂ６８

tert-ブチル *N*-{3-[4-(1-イソキノリルメチル)フェニル]プロピル}カルバメート

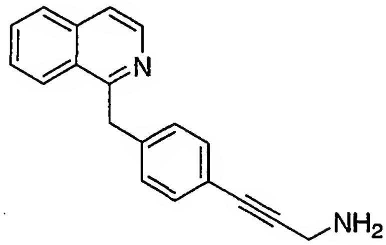


実施例Ｂ６７の化合物を実施例Ｂ４３と同様に処理し、表題化合物を得た。

[1]H-NMR($CDCl_3$)δ(ppm):1.43(9H, s), 1.70-1.81(2H, m), 2.54-2.60(2H, m), 3.01-3.20(2H, m), 4.47-4.57(1H, m), 4.65(2H, s), 7.07(2H, d), 7.21(2H, d), 7.55(1H, dd), 7.57(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.18(1H, d), 8.51(1H, d).

実施例Ｂ６９

3-[4-(1-イソキノリルメチル)フェニル]-2-プロピン-1-アミン



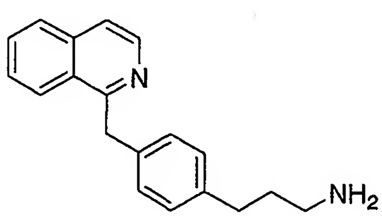
氷冷した実施例Ｂ６７の化合物4mgの塩化メチレン(0.6ml)溶液に、トリフルオロ酢酸0.3mlを加え、その温度で1時間攪拌した。反応混合物に飽和炭酸水素ナトリウム水溶液を加え、酢酸エチルで抽出し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物を4mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.60-3.68(2H, m),4.66(2H, s),7.19(2H, d), 7.29(2H, d), 7.53(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.82(1H, d), 8.10(1H, d), 8.49(1H, d).

アミンのプロトンは、NMRのチャート上観測されていない。

実施例Ｂ７０

3-[4-(1-イソキノリルメチル)フェニル]-1-プロパンアミン

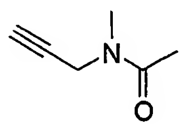


実施例Ｂ６８の化合物を実施例Ｂ６９と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.20-1.30(2H, m), 1.78-1.88(2H, m), 2.45-2.52(2H, m), 2.73-2.81(2H, m), 4.55(2H, s), 6.94(2H, d), 7.08(2H, d), 7.50(1H, dd), 7.51(1H, d), 7.61(1H, dd), 7.76(1H, d), 8.10(1H, d), 8.38(1H, d).

実施例Ｂ７１

N-メチル-*N*-(2-プロピニル)アセトアミド



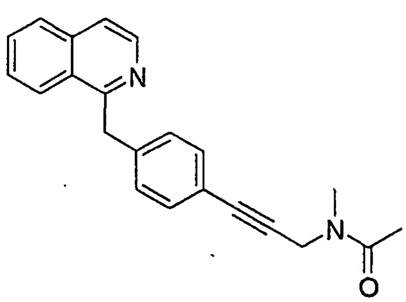
N-メチル-*N*-(2-プロピニル)アミンを実施例Ｂ５９と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.11(2.1H, s), 2.17(0.9H, s),2.21(0.7H, t), 2.31(0.3H, t), 3.00(0.9H, s), 3.08(2.1H, s), 4.04(0.6H, d), 4.23(1.4H, d).

なお、この化合物はアミド幾何異性体の7:3の混合物である。

実施例Ｂ７２

N-{3-[4-(1-イソキノリルメチル)フェニル]-2-プロピニル} *N*-メチルアセトアミド



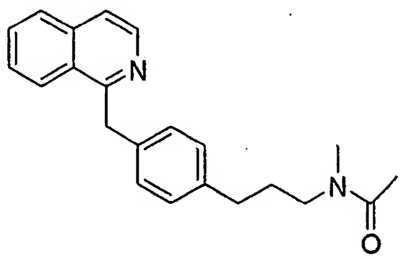
実施例Ｂ４１の化合物と実施例Ｂ７１の化合物を実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.10(1.8H, s), 2.11(1.2H, s), 3.01(1.2H, s), 3.10(1.8H, s), 4.21(1.2H, s), 4.41(0.8H, s), 4.67(2H, s), 7.18-7.23(2H, m), 7.29-7.32(2H, m), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

なお、この化合物はアミド幾何異性体の3:2の混合物である。

実施例Ｂ７３

N-{3-[4-(1-イソキノリルメチル)フェニル]プロピル}- N1-メチルアセトアミド



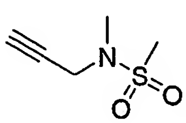
実施例Ｂ７２の化合物を実施例Ｂ４３と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.70-1.90(2H, m), 1.89(1.5H, s), 2.03(1.5H, s), 2.50-2.59(2H, m), 2.88(1.5H, s), 2.91(1.5H, s), 3.20-3.25(1H, m), 3.36-3.40(1H, m), 4.66(2H, s), 7.03-7.10(2H, m), 7.18-7.30(2H, m), 7.53(1H, dd), 7.58(1H, d), 7.66(1H, dd), 7.82(1H, d), 8.17(1H, d), 8.50(1H, d).

なお、この化合物はアミド幾何異性体の1:1の混合物である。

実施例Ｂ７４

N-メチル- *N*-(2-プロピニル)メタンスルホンアミド

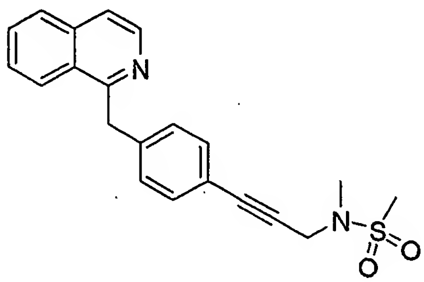


氷冷した*N*-メチル- *N*-(2-プロピニル)アミン2603mgの塩化メチレン(25ml)溶液に、トリエチルアミン6.55mlを加えた後、メタンスルホニルクロリド3.50mlを滴下後、その温度で1時間攪拌し、さらに室温で2時間攪拌した。反応混合物に氷を加え、酢酸エチルで抽出し、1規定塩酸、飽和炭酸水素ナトリウム水溶液そして飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥し後、シリカゲル濾過した。濾液を減圧濃縮し、表題化合物4522mgを得た。得られた化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR($CDCl_3$)δ(ppm):2.41(1H, t), 2.93(3H, s), 2.96(3H, s), 4.09(2H, d).

実施例Ｂ７５

N-{3-[4-(1-イソキノリルメチル)フェニル]-2-プロピニル}- *N*-メチルメタンスルホンアミド

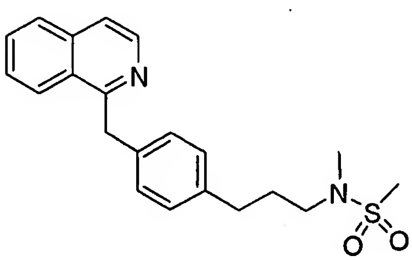


実施例Ｂ４１の化合物と実施例Ｂ７４の化合物を実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.95(3H, s), 2.97(3H, s), 4.26(2H, s), 4.68(2H, s), 7.24(2H, d), 7.31(2H, d), 7.55(1H, dd), 7.59(1H, d), 7.66(1H, dd), 7.83(1H, d), 8.10(1H, d), 8.49(1H, d).

実施例Ｂ７６

N-{3-[4-(1-イソキノリルメチル)フェニル]プロピル}- *N*-メチルメタンスルホンアミド



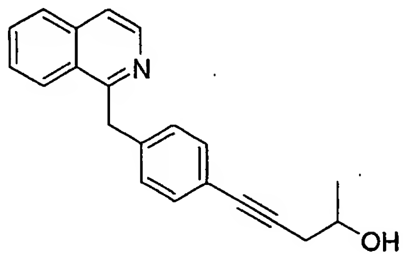
実施例Ｂ７５の化合物を実施例Ｂ４３と同様に反応させ、残査はLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液=1：99～100：0/20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM, 20mmΦx 50mm(long)]により分離精製

し、表題化合物を得た。

MS m/z(ESI:MH^+):369.2

実施例Ｂ７７

5-[4-(1-イソキノリルメチル)フェニル]-4-ペンチン-2-オール



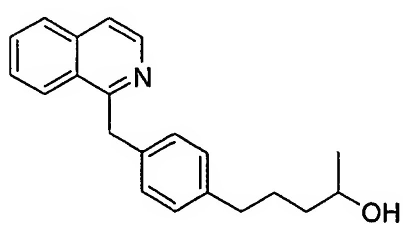
実施例Ｂ４１の化合物と4-ペンチン-2-オールを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.27(3H, t), 2.38-2.62(2H, m), 3.95-4.03(1H, m), 4.65(2H, s), 7.19(2H, d), 7.29(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.48(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ７８

5-[4-(1-イソキノリルメチル)フェニル]-2-ペンタノール

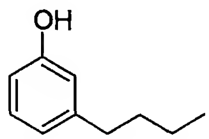


実施例Ｂ７７の化合物を実施例Ｂ４３と同様に反応させ、残査はLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液=1：99～100：0/20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM, 20mmΦx 50mm(long)]により分離精製し、表題化合物を得た。

MS m/z(ESI:MH^+):306.2

実施例Ｂ７９

3-ブチルフェノール

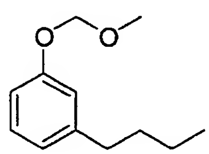


1-ブチル-3-メトキシベンゼンを実施例Ｂ４０と同様に処理し表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.94(3H, t), 1.30-1.55(2H, m), 1.55-1.62(2H, m), 2.56(2H, t), 4.76(1H, brs), 6.63(1H, dd), 6.66(1H, d), 6.75(1H, d), 7.12(1H, dd).

実施例Ｂ８０

1-ブチル-3-(メトキシメトキシ)ベンゼン

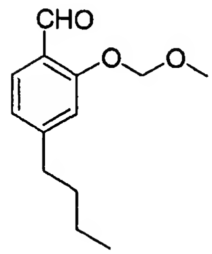


氷冷した実施例Ｂ７９の化合物318mgのジメチルホルムアミド(5ml)溶液に、60%鉱油分散の水素化ナトリウム102mgを加え、室温で30分間撹拌した。再度氷冷し、クロロメチルメチルエーテル0.18mlを加え、室温で12時間撹拌した。反応混合物に水を加え、酢酸エチルで抽出し、飽和炭酸水素ナトリウム水溶液と飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、シリカゲル濾過した。濾液を減圧濃縮し、表題化合物341mgを得た。得られた化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR($CDCl_3$)δ(ppm):0.94(3H, t), 1.30-1.42(2H, m), 1.55-2.04(2H, m), 2.58(2H, t), 3.49(3H, s), 5.17(2H, s), 6.80-6.87(3H, m), 7.18(1H, dd).

実施例Ｂ８１

4-ブチル-2-(メトキシメトキシ)ベンズアルデヒド

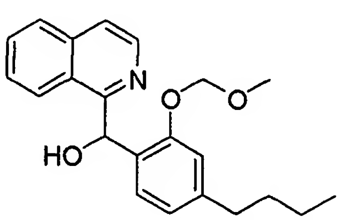


-20℃に冷却した実施例Ｂ８０の化合物2396mgの石油エーテル溶液に、t-ブチルリチウムのペンタン溶液(1.51M)10.6mlを滴下し、-10℃から0℃の温度範囲で1.5時間攪拌した。その反応溶液を-70℃に冷却し、無水エーテル17ml、ジメチルホルムアミド1.91mlを加え、その温度で3時間攪拌し、室温でさらに1時間攪拌した。反応混合物を氷冷し、飽和塩化アンモニウム水溶液を加え、酢酸エチルで抽出した。飽和食塩水で洗浄後、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物1821mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.94(3H, t), 1.32-1.42(2H, m), 1.57-1.65(2H, m), 2.64(2H, t), 3.54(3H, s), 5.29(2H, s), 6.91(1H, d), 7.01(1H, s),7.76(1H, d),10.44(1H, s).

実施例Ｂ８２

[4-ブチル-2-(メトキシメトキシ)フェニル](1-イソキノリル)メタノール



Org. Synth.,IV, 115(1988)の文献に基づいて合成した1-シアノ-ベンゾイル-1,2-ジヒドロイソキノリン815mg、実施例Ｂ８１の化合物869m

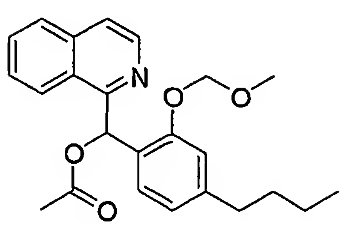
gそしてトリエチルベンジルアンモニウムクロリド7mgの塩化メチレン(1.6ml)溶液に、50%水酸化ナトリウム水溶液1.4mlを加え、10分間水浴中で超音波を照射した。反応混合物に塩化メチレン8.3mlとエタノール4.4mlを加え、さらに85分間水浴中で超音波を照射した。反応混合物に水を加え、塩化メチレンで抽出した。無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物1144mgを得た。

^{1}H-NMR(DMSO-d6)δ(ppm):0.86(3H, t), 1.22-1.31(2H, m), 1.44-1.52(2H, m), 2.44-2.51(2H, m), 3.16(3H, s), 5.10(1H, d), 5.12(1H, d), 6.72(1H, s), 6.75(1H, d), 6.84(1H, s), 7.21(1H, d), 7.61(1H, dd), 7.72(1H, dd), 7.74(1H, d), 7.95(1H, d), 8.31(1H, d), 8.42(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ８３

[4-ブチル-2-(メトキシメトキシ)フェニル](1-イソキノリル)メチルアセテート



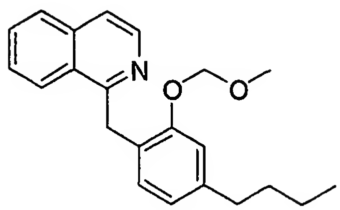
実施例Ｂ８２の化合物を実施例Ｂ３８と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.28-1.40(2H, m), 1.50-1.60(2H, m), 2.22(3H, s), 2.54(2H, t), 3.41(3H, s), 5.22(1H, d), 5.26(1H, d), 6.77(1H, d), 6.94(1H, s), 7.29(1H, d), 7.55(1H, dd), 7.58(1H, d), 7.70(1H, dd), 7.81(1H, d), 8.05(1H, s), 8.35(1H,

d), 8.55(1H, d).

実施例Ｂ８４

1-[4-ブチル-2-(メトキシメトキシ)ベンジル]イソキノリン

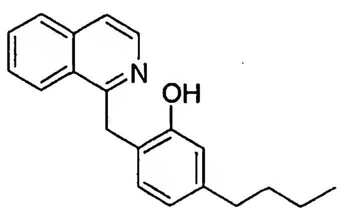


実施例Ｂ８３の化合物を実施例Ｂ３９と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.28-1.37(2H, m), 1.50-1.58(2H, m), 2.53(2H, t), 3.46(3H, s), 4.65(2H, s), 5.24(2H, s), 6.66(1H, dd), 6.89(1H, d), 6.92(1H, d), 7.51(1H, dd), 7.53(1H, d), 7.62(1H, dd), 7.79(1H, d), 8.23(1H, d), 8.47(1H, d).

実施例Ｂ８５

5-ブチル-2-(1-イソキノリルメチル)フェノール



実施例Ｂ８４の化合物88mgのメタノール(1.5ml)溶液に、5規定塩酸1.0mlを加え、室温で14時間撹拌した。5規定水酸化ナトリウム水溶液にて中和した後、リン酸緩衝液でpHを6.8に調整し酢酸エチルで抽出した。無水硫酸マグネシウムで乾燥後、減圧濃縮し、表題化合物44mgを得た。

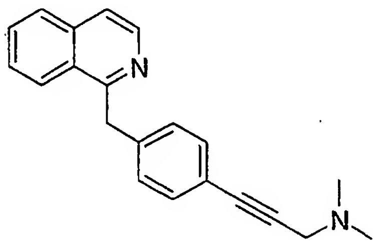
^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.23-1.37(2H, m), 1.48-1.60(2H, m), 2.51(2H, t), 4.56(2H, s), 6.65(1H, dd), 6.82(1H, d), 7.21(1H, d), 7.55(1H, d), 7.68(1H, dd), 7.72(1H, dd), 7.82(1H,

d), 8.35(1H, d), 8.44(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ８６

N-{3-[4-(1-イソキノリルメチル)フェニル]-2-プロピニル}-*N*,*N*-ジメチルアミン

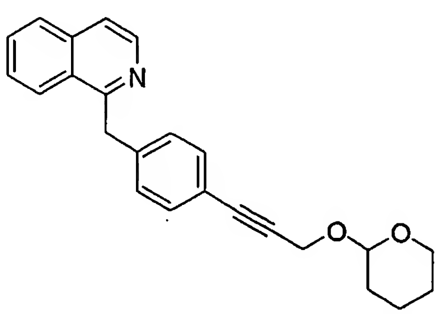


実施例Ｂ４１の化合物と1-ジメチルアミノ-2-プロピンを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.04(3H, s), 2.34(3H, s), 3.47(2H,s), 4.66(2H, s), 7.20(2H, d), 7.32(2H, d), 7.53(1H, dd), 7.56(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.10(1H, d), 8.50(1H, d).

実施例Ｂ８７

1-{4-[3-(テトラヒドロ-2H-2-ピラニルオキシ)-1-プロピニル]ベンジル}イソキノリン



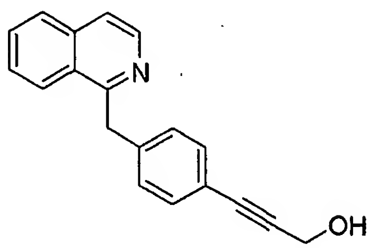
実施例Ｂ４１の化合物とテトラヒドロ-2-(2-プロピニルオキシ)-2H-ピランを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.45-1.85(6H, m),3.50-3.60(1H, m), 3.84-3.90(1H, m), 4.42(1H, d), 4.48(1H, d), 4.66(2H, 8), 4.87(1H, dd),

7.15-7.21(2H, m), 7.33-7.36(2H, m), 7.50-7.70(3H, m), 7.81-7.86(1H, m), 8.07-8.10(1H, m), 8.48-8.51(1H, m).

実施例Ｂ８８

3-[4-(1-イソキノリルメチル)フェニル]-2-プロピン-1-オール

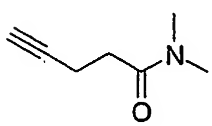


実施例Ｂ８７の化合物を実施例Ｂ４７と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.20-1.30(1H, m), 4.46(2H, s), 4.67(2H, s), 7.23(2H, d), 7.31(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.09(1H, d), 8.49(1H, d).

実施例Ｂ８９

N,N-ジメチル-4-ペンチンアミド

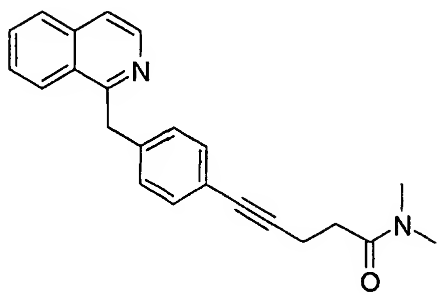


4-ペンチノイック酸552mgの塩化メチレン(150ml)溶液に、ジメチルアミン(2Mテトラヒドロフラン溶液)8.53ml、トリエチルアミン2.59mlそして1-(3-ジメチルアミノプロピル)-3-エチルカルボジイミド3221mgを加え、室温で24時間撹拌した。反応混合物を1規定塩酸、飽和炭酸水素ナトリウム水溶液、水そして飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮し、表題化合物129mg を得た。得られた化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR($CDCl_3$)δ(ppm):1.96-1.99(1H, m), 2.50-2.60(4H, m), 2.96(3H, s), 3.02(3H, s).

実施例B90

N,N-ジメチル-5-[4-(1-イソキノリルメチル)フェニル]-4-ペンチンアミド

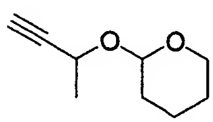


実施例B41の化合物と実施例B89の化合物を用いて、実施例B42と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.59-2.64(2H, m), 2.71-2.75(2H, m), 2.96(3H, s), 3.03(3H, s), 4.66(2H, s), 7.18(2H, d), 7.28(2H, d), 7.43-7.70(3H, m), 7.90(1H, d), 8.09(1H, d), 8.50(1H, d).

実施例B91

1-メチル-2-プロピニルテトラヒドロ-2H-2-ピラニルエーテル



3-ブチン-2-オール3051mgのジクロロメタン(150ml)溶液に、3,4-ジヒドロ-2H-ピラン7.15mlとピリジニウム*p*-トルエンスルホン酸2187mgを加え、室温で29時間撹拌した。

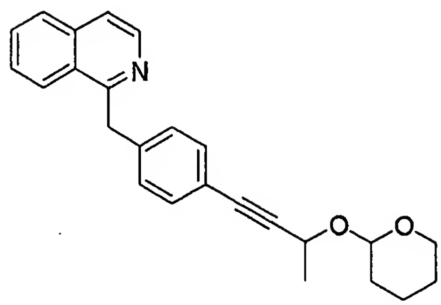
反応混合物を飽和炭酸水素ナトリウム水溶液、水そして飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物を4698mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 1.45(1.05H, d),1.48(1.95H, d), 1.50-1.90(6H, m), 2.37(0.65H, d), 2.43(0.35H, d), 3.50-3.60(1.3H, m), 3.80-3.86(0.7H, m),4.4-3-4.50(0.35H, m), 4.52-4.60(0.65H, m), 4.7

7(0.35H, t), 4.94(0.65H, t).

実施例Ｂ９２

1-{4-[3-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ブチニル]ベンジル}イソキノリン

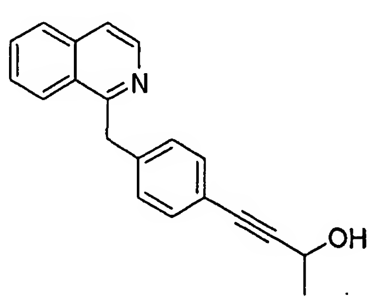


実施例Ｂ４１の化合物と実施例Ｂ９１の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.40-1.80(6H, m), 1.49(1.05H, d), 1.52(1.95H, d), 3.49-3.60(1H, m), 3.80-3.88(0.65H, m), 3.99-4.06(0.35H, m), 4.65(2H, s), 4.74(1H, q), 4.83(0.35H, t), 4.97(0.65H, t), 7.18-7.22(2H, m), 7.32(2H, d), 7.54(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.08(1H, d), 8.49(1H, d).

実施例Ｂ９３

4-[4-(1-イソキノリルメチル)フェニル]-3-ブチン-2-オール



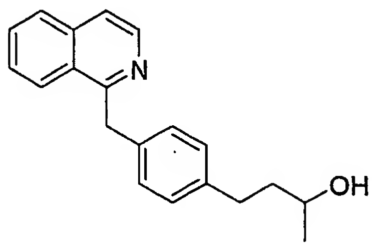
実施例Ｂ９２の化合物を実施例Ｂ４７と同様の方法で処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.53(3H, d), 2.15(1H, brs), 4.68(2H, s),

4.72(1H, q), 7.21(2H, d), 7.31(2H, d), 7.54(1H, dd), 7.59(1H, d), 7.66(1H, dd), 7.84(1H, d), 8.10(1H, d), 8.51(1H, d).

実施例Ｂ９４

4-[4-(1-イソキノリルメチル)フェニル]-2-ブタノール

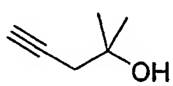


実施例Ｂ９３の化合物を実施例Ｂ４３と同様に反応させ、残査はLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液=1：99～100：0/20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM, 20mmΦx 50mm(long)]により分離精製し、表題化合物を得た。

MS m/z(ESI:MH^+):292.2

実施例Ｂ９５

2-メチル-4-ペンチン-2-オール



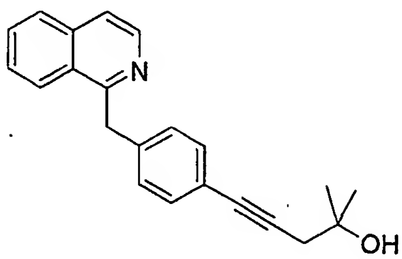
0℃に冷却したイソブチレンオキシド1889mgのテトラヒドロフラン(13ml)とジメチルスルホキシド(20ml)の混合溶液に、リチウムアセチリド-エチレンジアミン錯体を少しずつ加え、0℃にて5時間撹拌した。反応混合物に水を加え、酢酸エチルで抽出し、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、シリカゲルを用いて濾過した。濾液を減圧濃縮し、表題化合物3316mgを得た。このものはそれ以上精製することなく次反応に用いた。

^{1}H-NMR($CDCl_3$)δ(ppm):1.33(6H, s), 2.09(1H, t), 2.38(2H, t).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ９６

5-[4-(1-イソキノリルメチル)フェニル]-2-メチル-4-ペンチン-2-オール

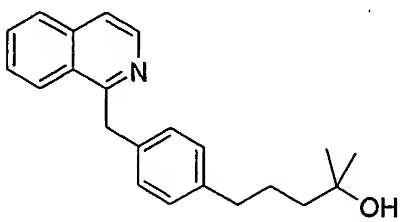


実施例Ｂ４１の化合物と実施例Ｂ９５の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR(DMSO-d6)δ(ppm):1.18(6H, s), 2.28(1H, s), 2.42(2H, s), 4.62(2H, s), 7.10-7.30(4H, m), 7.62(1H, dd), 7.71(1H, d), 7.72(1H, dd), 7.94(1H, d), 8.27(1H, d), 8.42(1H, d).

実施例Ｂ９７

5-[4-(1-イソキノリルメチル)フェニル]-2-メチル-2-ペンタノール

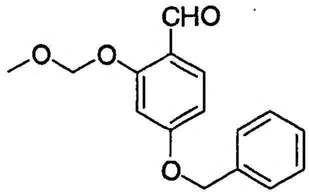


実施例Ｂ９６の化合物を実施例Ｂ４３と同様に反応させ、残査はLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液=1：99～100：0/20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM, 20mmΦx 50mm(long)]により分離精製し、表題化合物を得た。

MS m/z(ESI:MH^{+}):320.2

実施例Ｂ９８

4-ベンジルオキシ-2-(メトキシメトキシ)ベンズアルデヒド

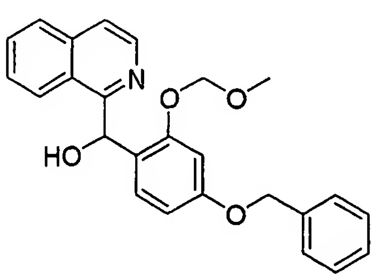


4-ベンジルオキシ-2-ヒドロキシベンズアルデヒド2071mgのテトラヒドロフラン(30ml)溶液に、*N*,*N*-ジイソプロピルエチルアミン1.98mlとクロロメチルメチルエーテル0.76mlを加え、加熱還流下19時間撹拌した。この反応溶液に*N*,*N*-ジイソプロピルエチルアミン2.7mlとクロロメチルメチルエーテル1.04mlを加え、加熱還流下さらに１０時間撹拌した。反応混合物に水を加え、酢酸エチルで抽出し、飽和塩化アンモニウム水溶液、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、シリカゲル及びアルミナを用いて濾過した。濾液を減圧濃縮し、表題化合物2470mg を得た。この化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR($CDCl_3$)δ(ppm):3.52(3H, s), 5.12(2H, s), 5.27(2H, s), 6.68(1H, dd), 6.80(1H, d), 7.33-7.45(5H, m), 7.82(1H, d), 10.33(1H, s).

実施例Ｂ９９

[4-(ベンジルオキシ)-2-(メトキシメトキシ)フェニル](1-イソキノリル)メタノール



実施例Ｂ９８の化合物を実施例Ｂ８２と同様に処理し、表題化合物を得た。

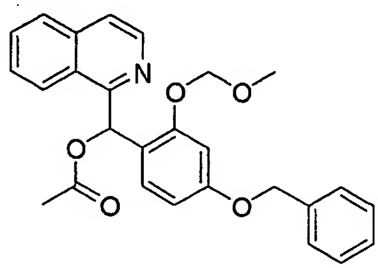
^{1}H-NMR(DMSO-d6)δ(ppm):3.16(3H, s), 5.01(2H, s), 5.11(1H, d),

5.14(1H, d), 6.59(1H, dd), 6.66-6.70(2H, m), 7.18(1H, d), 7.31(1H, d), 7.34-7.42(4H, m), 7.61(1H, dd), 7.71(1H, d), 7.75(1H, d), 7.95(1H, d), 8.28(1H, d), 8.43(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ１００

[4-(ベンジルオキシ)-2-(メトキシメトキシ)フェニル](1-イソキノリル)メチル アセテート

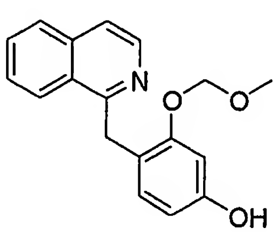


実施例Ｂ９９の化合物を実施例Ｂ３８と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.21(3H, s), 3.42(3H, s), 4.98(1H, d), 5.00(1H, d),5.21-5.27(2H, m), 6.54(1H, dd), 6.81(1H, d), 7.25(1H, d), 7.30-7.41(5H, m), 7.53(1H, dd), 7.57(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.00(1H, s), 8.29(1H, d), 8.55(1H, d).

実施例Ｂ１０１

4-(1-イソキノリルメチル)-3-(メトキシメトキシ)フェノール



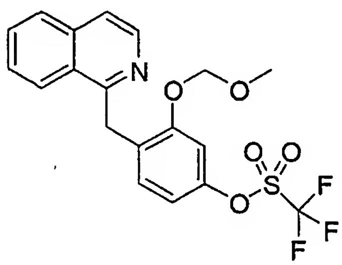
実施例Ｂ１００の化合物を実施例Ｂ３９と同様に処理し、表題化合物を得た。

^{1}H-NMR(DMSO-d6)δ(ppm):3.36(3H, s), 4.44(2H, s), 5.17(2H, s),

6.22(1H, d), 6.52(1H, s), 6.67(1H, d), 7.57-7.76(3H, m), 7.92(1H, d), 8.22(1H, d), 8.37(1H, d), 9.24(1H, brs).

実施例Ｂ１０２

4-(1-イソキノリルメチル)-3-(メトキシメトキシ)フェニルトリフルオロメタンスルホネート

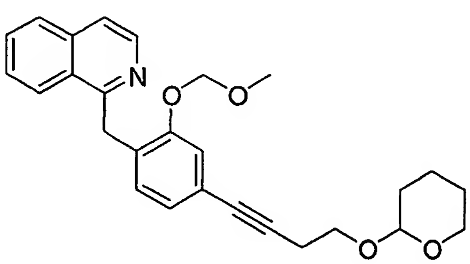


実施例Ｂ１０１の化合物を実施例Ｂ４１と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.43(3H, s), 4.65(2H, s), 5.24(2H, s), 6.77(1H, dd), 7.04(1H, d), 7.07(1H, d), 7.54-7.61(2H, m), 7.67(1H, dd), 7.84(1H, d), 8.16(1H, d), 8.47(1H, d).

実施例Ｂ１０３

1-{2-(メトキシメトキシ)-[4-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ブチニル]ベンジル}イソキノリン



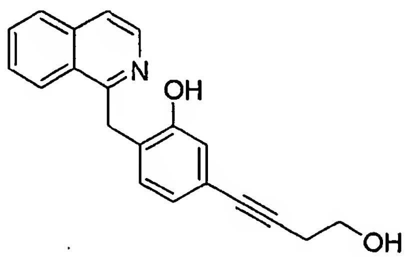
実施例Ｂ１０２の化合物と2-(3-ブチニルオキシ)テトラヒドロ-2H-ピランを用い、実施例Ｂ４２と同様に処理して表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.51-1.90(6H, m), 2.68(2H, t), 3.50(3H, s), 3.49-3.55(1H, m), 3.58-3.65(1H, m), 3.84-3.94(2H, m), 4.63-4.68(1H, m), 4.65(2H, s), 5.23(2H, s), 6.76(1H, dd), 7.04(1H,

d), 7.07(1H, d), 7.49-7.69(3H, m), 7.81(1H, d), 8.14(1H, d), 8.47(1H, d).

実施例B104

5-(4-ヒドロキシ-1-ブチニル)-2-(1-イソキノリルメチル)フェノール



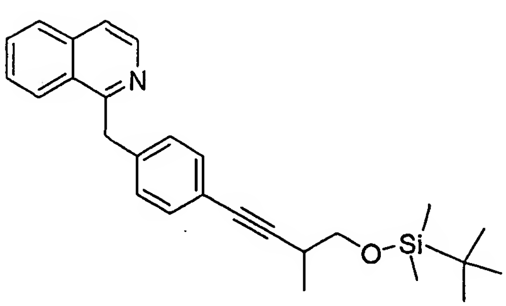
実施例B103の化合物を実施例B85と同様に処理して表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):1.80(1H, brs), 2.66(2H, t), 3.73-3.82(2H, m), 4.58(2H, s), 6.87(1H, d), 7.04(1H, s), 7.23(1H, d), 7.60(1H, d), 7.69-7.78(2H, m), 7.86(1H, d), 8.37(1H, d), 8.42(1H, d).

フェノール性水酸基のプロトンは、NMRのチャート上観測されていない。

実施例B105

1-(t-ブチル)-1,1-ジメチルシリル{4-[4-(1-イソキノリルメチル)フェニル]-2-メチル-3-ブチニル}エーテル



氷冷した四臭化炭素11.19gの塩化メチレン(60ml)溶液に、トリフェニルフォスフィン18.37gを加え、その温度で1時間攪拌した。この溶液にTetrahedron Lett.,4347 (1979)の文献に基づいて合成した3-{[1-(t-ブ

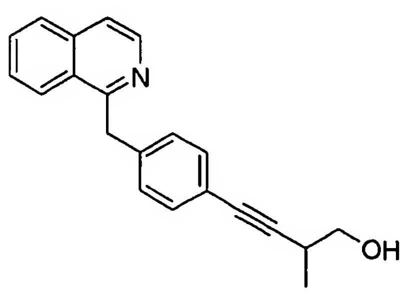
チル)-1,1-ジメチルシリル]オキシ}-2-メチルプロパナールの塩化メチレン溶液(14ml)を滴下し、さらに1時間撹拌した。反応混合物を塩化メチレンで希釈し、飽和炭酸水素ナトリウム水溶液、飽和塩化アンモニウム水溶液そして飽和食塩水で洗浄し、硫酸マグネシウムで乾燥後、減圧濃縮した。このものにエーテルを加え不溶物を濾別し、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、t-ブチル[(4,4-ジブロモ-2-メチル-3-ブテニル)オキシ]ジメチルシラン2385mgを得た。

次いで、-70℃に冷却したt-ブチル[(4,4-ジブロモ-2-メチル-3-ブテニル)オキシ]ジメチルシラン1326mgのテトラヒドロフラン(10ml)溶液にn-ブチルリチウム2.47Mヘキサン溶液3.15mlを滴下し、その温度で1時間撹拌した。飽和塩化アンモニウム水溶液を加え、室温に昇温した。反応混合物に水を加え、エーテルで抽出した。エーテル層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、シリカゲル濾過し、濾液を減圧濃縮した。得られた残渣と実施例Ｂ４１の化合物を実施例Ｂ４２と同様に処理して、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.07(6H, s), 0.90(9H, s), 1.18(3H, d),2.70-2.80(1H, m), 3.47(1H, dd), 3.70(1H, dd), 4.65(2H, s), 7.16(2H, d), 7.27(2H, d), 7.51(1H, dd), 7.56(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.07(1H, d), 8.49(1H, d).

実施例Ｂ１０６

4-[4-(1--イソキノリルメチル)フェニル]-2-メチル-3-ブチン-1-オール

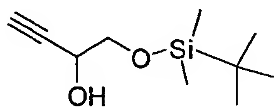


実施例Ｂ１０５の化合物を実施例Ｂ４７と同様に処理して表題化合物を得た。

[1]H-NMR(DMSO-d6)δ(ppm):1.11(3H, d), 2.60-2.70(1H, m), 3.28(1H, d), 3.44(1H, d), 4.58(2H, s), 4.85-4.90(1H, m), 7.23(4H, s), 7.61(1H, dd), 7.70(1H, d), 7.71(1H, dd), 7.93(1H, d), 8.25(1H, d), 8.42(1H, d).

実施例Ｂ１０７

1-{[1-(t-ブチル)-1,1-ジメチルシリル]オキシ}-3-ブチン-2-オール

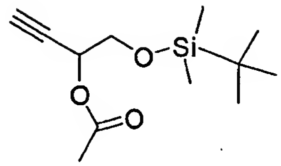


窒素雰囲気下、無水テトラヒドロフラン20mlを-78℃に冷却し、エチニルマグネシウムブロミド0.5モルのテトラヒドロフラン溶液90mlを加えた。この溶液にt-ブチルジメチルシロキシアセトアルデヒド6000mgのテトラヒドロフラン溶液(30ml)を滴下した。-78℃で45分間、室温に昇温し1時間40分攪拌した。反応混合物を氷冷し、飽和塩化アンモニウム水溶液を加え、エーテルで抽出し、水と飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、シリカゲルを用いて濾過した。濾液を減圧濃縮し、表題化合物8.55gを得た。この化合物はさらに精製することなく次反応に用いた。

[1]H-NMR($CDCl_3$)δ(ppm):0.08(6H, s), 0.91(9H, s), 2.43 (1H, d), 2.60-2.66(1H, m), 3.65-3.70(1H, m), 3.73-3.81(1H, m), 4.38-4.42(1H, m).

実施例Ｂ１０８

1-{[1-(t-ブチル)-1,1-ジメチルシリル]オキシ}メチル)-2-プロピニル アセテート

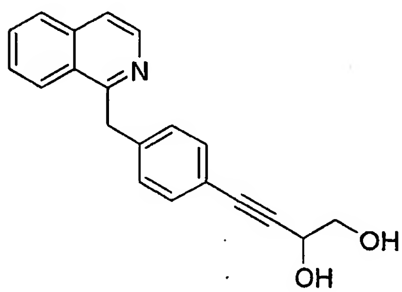


実施例Ｂ１０７の化合物を実施例Ｂ３８と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.08(6H, s), 0.90(9H, s), 2.11(3H, s), 2.44(1H,d), 3.80-3.88(2H, m), 5.41-5.55(1H, m).

実施例Ｂ１０９

4-[4-(1-イソキノリルメチル)フェニル]-3-ブチン-1,2-ジオール

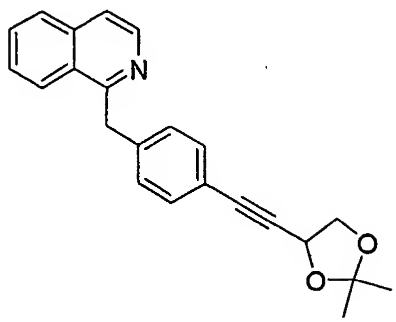


実施例Ｂ４１の化合物と実施例Ｂ１０８の化合物を用い、実施例Ｂ４２と同様に処理し、カップリング成績体を得た。さらにその成績体を実施例Ｂ４７と同様に水酸基保護基を脱保護し、表題化合物を得た。

^{1}H-NMR(DMSO-d6)δ(ppm):3.40-3.45(1H, m), 3.70-3.82(1H, m), 4.30-4.35(1H, m), 4.63(2H, s), 4.90(1H, t), 5.46(1H, d), 7.25-7.30(4H, m), 7.62(1H, dd), 7.71(1H, d), 7.73(1H, dd), 7.94(1H, d), 8.28(1H, d), 8.43(1H, d).

実施例Ｂ１１０

1-{4-[2-(2,2-ジメチル-1,3-ジオキソラン-4-イル)-1-エチニル]ベンジル}イソキノリン

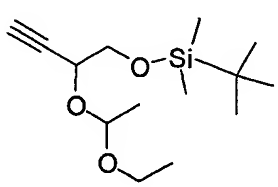


実施例Ｂ１０９の化合物34mgのジメチルホルムアミド(2ml)溶液に、2,2-ジメトキシプロパン0.36ml、10-カンファースルホン酸43mgそしてモレキュラシーブ4Åを加え、75℃で9時間撹拌した。反応混合物に飽和炭酸ナトリウム水溶液を加え、酢酸エチルで抽出し、水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物を14mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.40(3H, s), 1.50(3H, s), 3.97(1H, dd), 4.21(1H, dd), 4.66(2H, s), 4.91(1H, dd), 7.19(2H, d), 7.32(2H, d), 7.52(1H, dd), 7.65-7.78(2H, m), 8.08(1H, d), 8.09(1H, d), 8.49(1H, d).

実施例Ｂ１１１

t-ブチル{[2-(1-エトキシエトキシ)-3-ブチニル]オキシ}ジメチルシラン



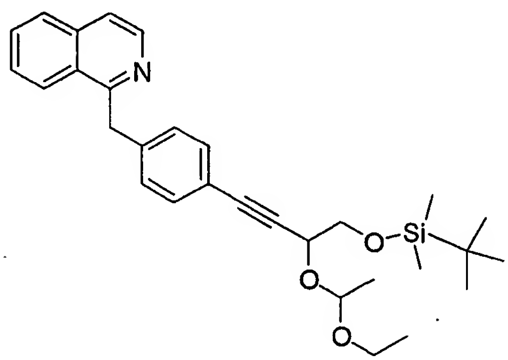
1-{[1-(t-ブチル)-1,1-ジメチルシリル]オキシ}-3-ブチン-2-オール1687mgの塩化メチレン(90ml)溶液に、エチルビニルエーテル1.21mlとピリジニウム*p*-トルエンスルホン酸塩317mgを加え、室温で1時間撹拌した。塩化メチレン層を飽和炭酸水素ナトリウム水溶液と飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮し、表題化合物1962mgを得た。

この化合物はさらに精製することなく次反応に用いた。

[1]H-NMR(DMSO-d6)δ(ppm):0.00(6H, s), 0.81(9H, s), 1.01-1.07(3H, m), 1.10-1.20(1H, m), 1.18(3H, d), 3.35-3.63(4H, m), 4.18-4.27(1H, m), 4.74(0.5H, q), 4.81(0.5H, q).

実施例Ｂ１１２

1-{4-[4-{[1-(t-ブチル)-1,1-ジメチルシリル]オキシ}-3-(1-エトキシエトキシ) -1-ブチニル]ベンジル}イソキノリン

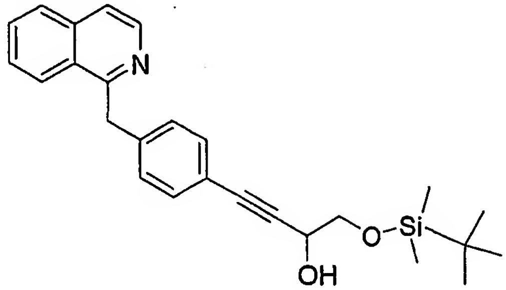


実施例Ｂ４１の化合物と実施例Ｂ１１１の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

[1]H-NMR(DMSO-d6)δ(ppm):0.00(6H, s), 0.80(9H, s), 1.01-1.05(3H, m), 1.19(3H, d), 3.39-3.70(4H, m), 4.41(0.5H, t), 4.48(0.5H, t), 4.59(2H, s), 4.79(0.5H, q), 4.87(0.5H, q), 7.20-7.30(4H, m), 7.58(1H, dd), 7.68(1H, d), 7.69(1H, dd), 7.91(1H, d), 8.24(1H, d), 8.38(1H, d).

実施例Ｂ１１３

1-{[1-(t-ブチル)-1,1-ジメチルシリル]オキシ}4-[4-(1-イソキノリルメチル)フェニル]-3-ブチン-2-オール

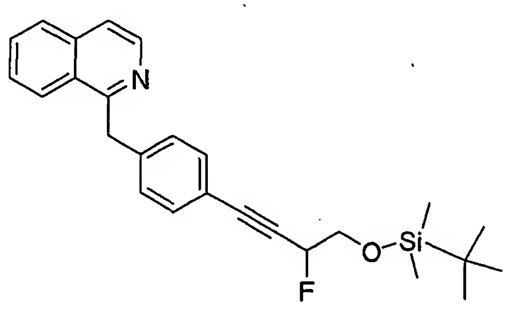


実施例Ｂ１１２の化合物474mgのメタノール(15ml)溶液に、ピリジニウム*p*-トルエンスルホン酸塩486mgを加え、室温で24時間攪拌した。反応混合物に酢酸エチルを加え、飽和炭酸水素ナトリウム水溶液と飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物265mgを得た。

^{1}H-NMR(DMSO-d6)δ(ppm):0.01(6H, s), 0.82(9H, s), 3.55-3.62(2H, m), 4.30-4.39(1H, m), 4.61(2H, s), 5.51(1H, d), 7.20-7.27(4H, m), 7.50-7.63(1H, m), 7.67-7.74(2H, m), 7.92(1H, d), 8.27(1H, d), 8.41(1H, d).

実施例Ｂ１１４

1-(t-ブチル)- 1,1- ジメチルシリル{2-フルオロ-4-[4-(1-イソキノリルメチル)フェニル] -3-ブチニル}エーテル



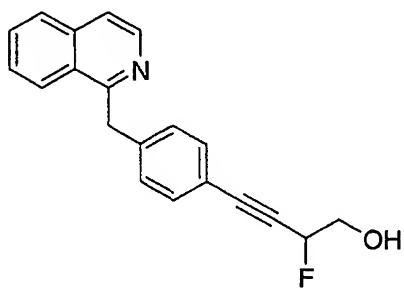
窒素雰囲気下、-78℃に冷却した(ジエチルアミノ)サルファートリフルオリド44μlの塩化メチレン(2ml)溶液に、実施例Ｂ１１３の化合物116mgの塩化メチレン溶液(2ml)を滴下し、15分間攪拌後、室温でさらに8時間攪拌した。反応混合物に飽和炭酸水素ナトリウム水溶液を加え、塩化メ

チレンで抽出した。塩化メチレン層を水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物42mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.10(6H, s), 0.91(9H, s), 3.83-4.00(2H, m), 4.67(2H, s), 5.17(1H, ddd), 7.22(2H, d), 7.34(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.08(1H, d), 8.50(1H, d).

実施例Ｂ１１５

2-フルオロ-4-[4-(1-イソキノリルメチル)フェニル]-3-ブチン-1-オール

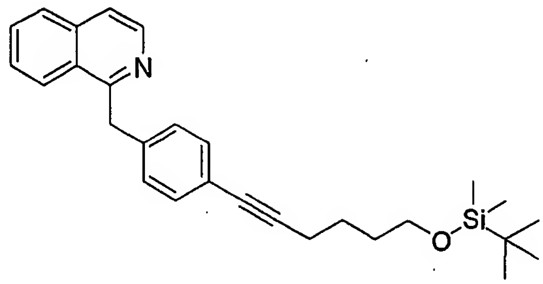


実施例Ｂ１１４の化合物を実施例Ｂ４７と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.31(1H, brs), 3.77-3.95(2H, m), 4.67(2H, s), 5.35(1H, ddd), 7.22(2H, d), 7.35(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.07(1H, d), 8.50(1H, d).

実施例Ｂ１１６

1-(t-ブチル)- 1,1-ジメチルシリル{6- [4-(1-イソキノリルメチル)フェニル]-5-ヘキシニル}エーテル

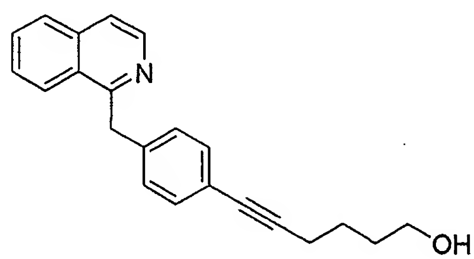


実施例Ｂ４１の化合物とt-ブチル(5-ヘキシニルオキシ)ジメチルシランを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.04(6H, s), 0.88(9H, s), 1.55-1.70(4H, m), 2.39(2H, t), 3.64(2H, t), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.51(1H, dd), 7.55(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.08(1H, d), 8.49(1H, d).

実施例Ｂ１１７

6-[4-(1-イソキノリルメチル)フェニル]-5-ヘキシン-1-オール



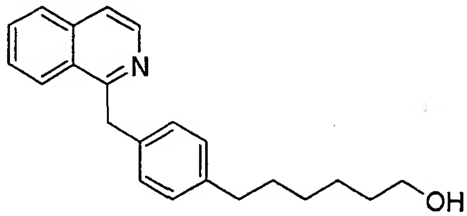
実施例Ｂ１１６の化合物実施例Ｂ４７と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.60-1.80(4H, m), 2.42(2H, t), 3.69(2H, t), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.49(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ１１８

6-[4-(1-イソキノリルメチル)フェニル]-1-ヘキサノール

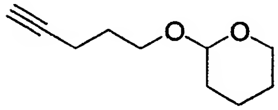


実施例Ｂ１１７の化合物を実施例Ｂ４３と同様に反応させ、残査はLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液=1：99～100：0/20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM, 20mmΦx 50mm(long)]により分離精製し、表題化合物を得た。

MS m/z(ESI:MH^+):320.2

実施例Ｂ１１９

2-(4-ペンチニルオキシ)テトラヒドロ-2H-ピラン

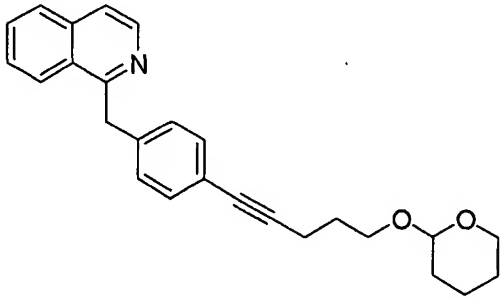


4-ペンチン-1-オールを実施例Ｂ９１と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.50-1.90(8H, m), 1.95(1H, t), 2.30-2.35(2H, m), 3.46-3.54(2H, m), 3.80-3.90(2H, m), 4.60(1H, dd).

実施例Ｂ１２０

1-{4-[5-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ペンチニル]ベンジル}イソキノリン

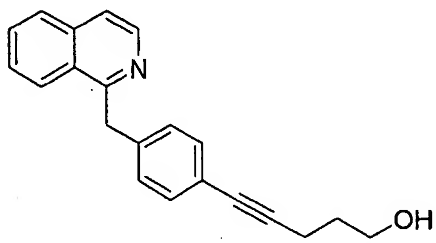


実施例Ｂ４１の化合物と実施例Ｂ１１９の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.49-1.90(8H, m), 2.49(2H, t), 3.47-3.54(2H, m), 3.82-3.90(2H, m), 4.60(1H, dd), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.52(1H, dd), 7.58(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

実施例Ｂ１２１

5-[4-(1-イソキノリルメチル)フェニル]-4-ペンチン-1-オール



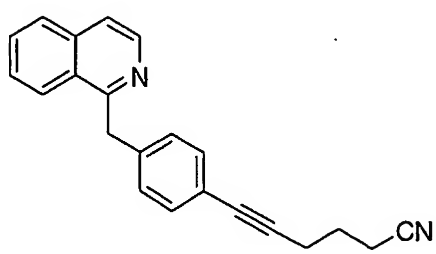
実施例Ｂ１２０の化合物を実施例Ｂ４７と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.80-1.88(2H, m), 2.51(2H, t), 3.80(2H, t), 4.65(2H, s), 7.18(2H, d), 7.29(2H, d), 7.52(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ１２２

5-[4-(1-イソキノリルメチル)フェニル]-4-ペンチニルシアニド

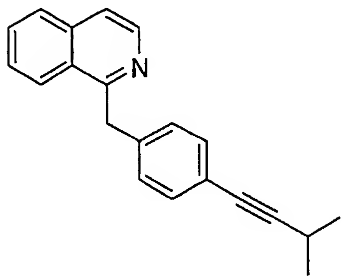


実施例Ｂ４１の化合物と5-シアノ-1-ペンチンを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.85-1.98(2H, m), 2.40-2.60(4H, m), 4.66(2H, s), 7.20(2H, d), 7.28(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.09(1H, d), 8.50(1H, d).

実施例Ｂ１２３

1-[4-(3-メチル-1-ブチニル)ベンジル]イソキノリン

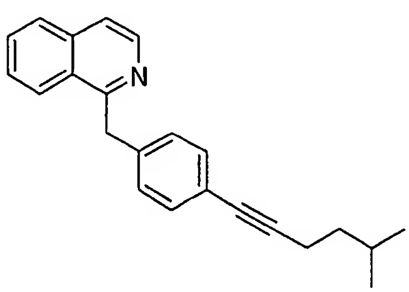


実施例Ｂ４１の化合物と3-メチル-1-ブチンを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.23(6H, d), 2.70-2.78(1H, m), 4.65(2H, s), 7.18(2H, d), 7.28(2H, d), 7.51(1H, dd), 7.58(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.08(1H, d), 8.50(1H, d).

実施例Ｂ１２４

1-[4-(5-メチル-1-ヘキシニル)ベンジル]イソキノリン



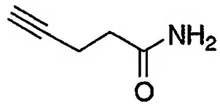
実施例Ｂ４１の化合物と5-メチル-1-ヘキシンを用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.91(6H, d), 1.47(2H, dt), 1.68-1.77(1H, m), 2.37(2H, t), 4.65(2H, s), 7.17(2H, d), 7.28(2H, d), 7.52(1

H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.09(1H, d), 8.49(1H, d).

実施例B125

4-ペンチンアミド

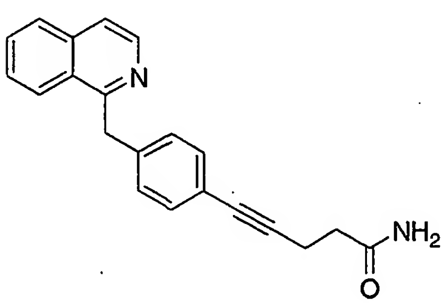


4-ペンチノイック酸2446mgのクロロホルム(75ml)溶液に、1-エトキシカルボニル-2-エトキシ-1,2-ジヒドロキノリン6775mgと炭酸水素アンモニウム5905mgを加え、室温で17.5時間攪拌した。反応混合物をセライトを用いて濾過し、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物を249mgを得た。

^{1}H-NMR(DMSO-d6)δ(ppm):2.21(2H, t), 2.29-2.33(2H, m), 2.73(1H, t), 6.78-6.88(1H, m), 7.28-7.38(1H, m).

実施例B126

5-[4-(1-イソキノリルメチル)フェニル]-4-ペンチンアミド

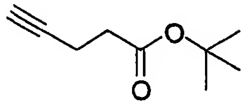


実施例B41の化合物と実施例B125の化合物を用いて、実施例B42と同様に処理し、表題化合物を得た。

^{1}H-NMR(DMSO-d6)δ(ppm):2.51(2H, t), 2.85(2H, t), 3.70(2H, brs), 4.59(2H, s), 7.05(2H, d), 7.23(2H, d), 7.61(1H, dd), 7.70(1H, d), 7.72(1H, dd), 7.94(1H, d), 8.30(1H, d), 8.43(1H, d).

実施例B127

t-ブチル4-ペンチノエート

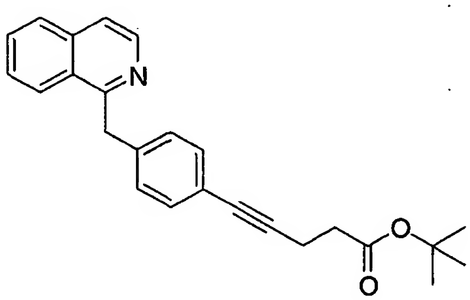


4-ペンチノイックアシッド2550mgの*N,N*-ジメチルアセトアミド(230ml)溶液に、ベンジルトリエチルアンモニウムクロリド5.92g、炭酸カリウム93.4gそしてt-ブチルブロミド143mlを加え、55℃にて24時間攪拌した。反応混合物に水を加え、酢酸エチルで抽出し、水で洗浄し、無水硫酸マグネシウムで乾燥後、シリカゲルを用いて濾過した。濾液を減圧濃縮し、表題化合物2.10gを得た。この化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR($CDCl_3$)δ(ppm):1.46(9H, s), 1.96-1.97(1H, m), 2.45-2.47(4H, m).

実施例Ｂ１２８

t-ブチル5-[4-(1-イソキノリルメチル)フェニル]-4-ペンチノエート

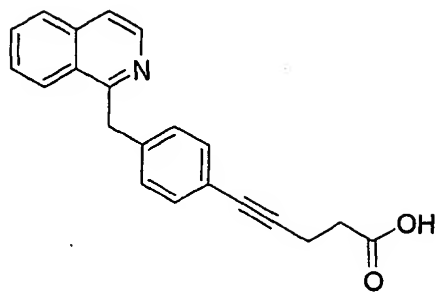


実施例Ｂ４１の化合物と実施例Ｂ１２７の化合物を用いて、実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.45(9H, s), 2.49(2H, t), 2.64(2H, t), 4.64(2H, s), 7.21(2H, d), 7.26(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

実施例Ｂ１２９

5-[4-(1-イソキノリルメチル)フェニル]-4-ペンチノイック酸



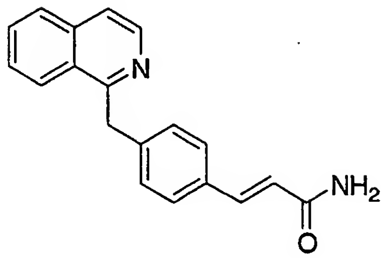
実施例Ｂ１２８の化合物を実施例Ｂ６９と同様に反応させ、残査はLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液=1：99～100：0/20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM, 20mmΦx 50mm(long)]により分離精製し、表題化合物を得た。

MS m/z(ESI:MH^+):316.1

以下の実施例の化合物は次の様に合成した。即ち実施例Ｂ３３に従い、実施例Ｂ４１の化合物と以下の各種反応剤を反応させ表題化合物を得た。各種反応剤とはアクリルアミド、*N*,*N*-ジメチルアクリルアミド、アクリル酸t-ブチルエステル、メチルビニルスルホンである。またその様にして得られたカップリング成績体を実施例Ｂ３９に従い還元するか、または実施例Ｂ４０に従いt-ブチルエステルを脱保護するか、またはその両方を行った。精製はシリカゲルカラムクロマトグラフィーもしくはLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液=1：99～100：0/20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM, 20mmΦx 50mm(long)]により行った。

実施例Ｂ１３０

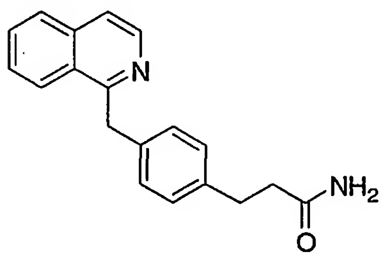
(E)-3-[4-(1-イソキノリルメチル)フェニル]-2-プロペンアミド



MS m/z(ESI:MH+):289.3

実施例Ｂ１３１

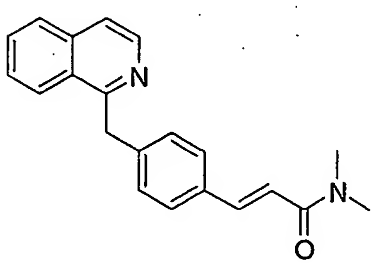
3-[4-(1-イソキノリルメチル)フェニル]-2-プロパンアミド



MS m/z(ESI:MH+):291.2

実施例Ｂ１３２

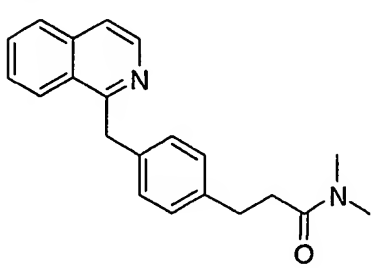
N,N-ジメチル-(E)- 3-[4-(1-イソキノリルメチル)フェニル]-2-プロペンアミド



MS m/z(ESI:MH+):317.3

実施例Ｂ１３３

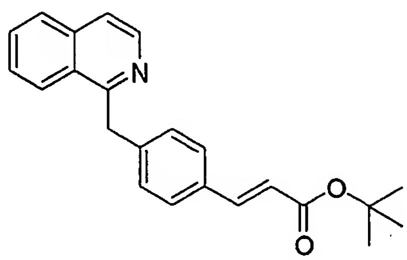
N,N-ジメチル3-[4-(1-イソキノリルメチル)フェニル]プロパンアミド



MS m/z(ESI:MH$^+$):319.1

実施例B134

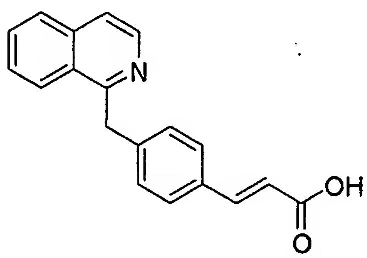
t-ブチル(E)-3-[4-(1-イソキノリルメチル)フェニル]-2-プロペノエート



^{1}H-NMR($CDCl_3$)δ(ppm):1.51(9H, s), 4.68(2H, s), 6.28(1H, d), 7.27(2H, d), 7.39(2H, d), 7.49-7.60(3H, m), 7.65(1H, dd), 7.82(1H, d), 8.11(1H, d), 8.50(1H, d).

実施例B135

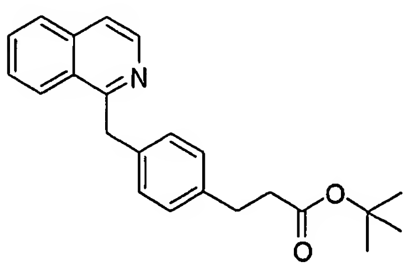
(E)-3-[4-(1-イソキノリルメチル)フェニル]-2-プロペノイック酸



MS m/z(ESI:MH$^+$):290.2

実施例B136

t-ブチル 3-[4-(1-イソキノリルメチル)フェニル]プロパノエート

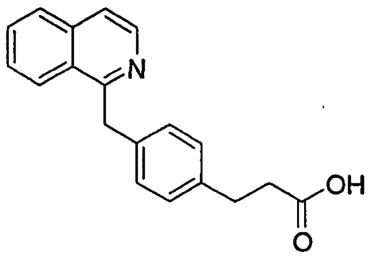


^{1}H-NMR($CDCl_3$)δ(ppm):1.37(9H, s), 2.47(2H, t), 2.83(2H, t), 4.64(2H, s), 7.07(2H, d), 7.19(2H, d), 7.52(1H, dd), 7.56(1H, d),

7.63(1H, dd), 7.81(1H, d), 8.14(1H, d), 8.49(1H, d).

実施例Ｂ１３７

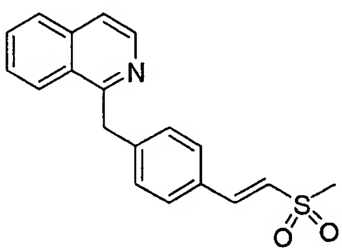
3-[4-(1-イソキノリルメチル)フェニル]プロパノイック酸



MS m/z(ESI:MH⁺):292.1

実施例Ｂ１３８

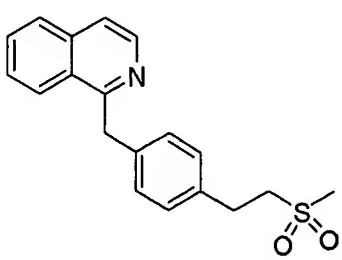
(E)-2-[4-(1-イソキノリルメチル)フェニル]-1-エテニルメチルスルホン



MS m/z(ESI:MH⁺):324.1

実施例Ｂ１３９

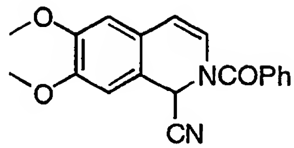
1-{4-[2-(メチルスルホニル)エチル]ベンジル}イソキノリン



MS m/z(ESI:MH⁺):326.1

実施例Ｂ１４０

2-ベンゾイル-6,7-ジメトキシ-1,2-ジヒドロ-1-イソキノリンカルボニトリル

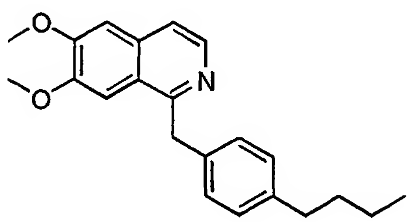


Tetrahedron, 37(23), 3977(1981)に基づいて合成した6,7-ジメトキシイソキノリン1.0g（5.3ミリモル）の塩化メチレン（6.0ml）溶液にシアン化カリウム1.0g（16ミリモル）水溶液(2.3ml)と塩化ベンゾイル1.1ml（9.5ミリモル）を加え、加熱還流下２時間攪拌した。室温まで戻した後、セライトを用いて濾過を行い、塩化メチレンと水で洗浄した。得られた濾液を分離し、塩化メチレン層を水、２規定塩酸、水そして２規定水酸化ナトリウムで洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物573mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.92(3H, s), 3.94(3H, s), 5.99(1H, d), 6.51-6.55(2H, m), 6.73(1H, s), 6.85(1H, s), 7.45-7.49(2H, m), 7.53-7.56(1H, m), 7.58-7.61(2H, m)

実施例Ｂ１４１

1-(4-ブチルベンジル)-6,7-ジメトキシイソキノリン



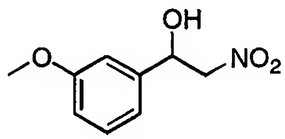
実施例Ｂ１４０の化合物と実施例Ｂ１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.27-1.36(2H, m), 1.51-1.58(2H, m), 2.54(2H, t), 3.88(3H, s), 4.01(3H, s), 4.57(2H, s), 7.05(1H, s), 7.07(2H, d), 7.19(2H, d), 7.32(1H, s), 7.43(1H, d),

8.37(1H, d)

実施例Ｂ１４２

1-(3-メトキシフェニル)-2-ニトロ-1-エタノール

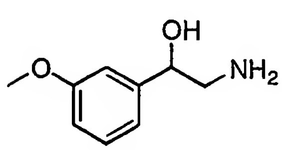


m-アニスアルデヒド5.0g（37ミリモル）とニトロメタン4.0ml（73ミリモル）のメタノール（50ml）溶液に、水酸化ナトリウム水溶液（水酸化ナトリウム1.5g（37ミリモル）を水15mlに溶解した。）を、溶液の温度が３０℃を越えないように滴下した。その後、室温で４時間撹拌した。氷冷後、酢酸水溶液（氷酢酸（37ミリモル）を水250mlに溶解した。）を反応溶液に加え、酢酸エチルで抽出した。酢酸エチル層を、水と５％炭酸水素ナトリウム水溶液で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物6.09g を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.83(3H, s), 4.52(1H, dd), 4.61(1H, dd), 4.76-4.78(1H, m), 5.44-5.48(1H, m), 6.90(1H, dd), 6.96-6.98(2H, m), 7.25-7.34(1H, m)

実施例Ｂ１４３

2-アミノ-1-(3-メトキシフェニル)-1-エタノール

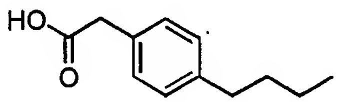


実施例Ｂ１４２の化合物3.0g（15ミリモル）のテトラヒドロフラン（43ml）とメタノール（43ml）の混合溶液に、パラジウム－炭素（10%）0.64gとギ酸アンモニウム4.8gを加え、室温で１８時間撹拌した。触媒を濾

去した後、濾液をエーテルで希釈し析出物を濾去し、得られた濾液を濃縮し、表題化合物を1.82g 得た。この化合物はさらに精製することなく次の反応に用いた。

実施例Ｂ１４４

2-(4-ブチルフェニル)アセティックアシッド



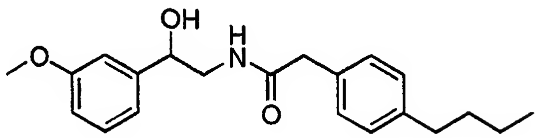
4-n-ブチルベンジルアルコール9.6g（59ミリモル）のエーテル（120ml）溶液に塩化チオニル4.7ml（66ミリモル）を滴下し、室温で２時間撹拌した。減圧下、溶媒を除去し、過剰の塩化チオニルをベンゼンで共沸することにより除去した。残渣のジメチルスルフォキサイド（50ml）溶液に、シアン化ナトリウム86g（1.8モル）とヨウ化n-テトラブチルアンモニウム2.2g（5.9ミリモル）を加え、室温で１６時間撹拌した。水を加え、酢酸エチルで抽出した。酢酸エチル層を、水と飽和食塩水で洗浄後、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、n-ブチルフェニルアセトニトリル8.2gを黄色油状物として得た。次に、水58mlに濃硫酸48mlを滴下し、その温度を５０℃まで冷却した。その溶液に、上記で得られたn-ブチルフェニルアセトニトリル8.2gを滴下し、加熱還流下１６時間撹拌した。室温まで冷却後、析出した結晶をろ取し、水で洗浄した。その結晶を0.1規定の水酸化ナトリウム水溶液（200ml）に溶解し、Norit5gを加え、還流下２時間撹拌した。セライトを用いてNoritを濾去し、濾液を室温まで冷却後、1規定塩酸を用いて濾液を酸性にすることにより、結晶が析出した。析出した結晶をろ取し、水で洗浄し、結晶を乾燥後、表題化合物3.5gを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.93(3H, t), 1.30-1.40(2H, m), 1.53-1.62(2H, m), 2.59(2H, t), 3.62(2H, s), 7.15(2H, d), 7.20(2H, d)

但し、カルボキシル基のOHはNMRのチャート上は見えていない。

実施例Ｂ１４５

N-[2-ヒドロキシ-2-(3-メトキシフェニル)エチル]-2-(4-ブチルフェニル)アセタミド

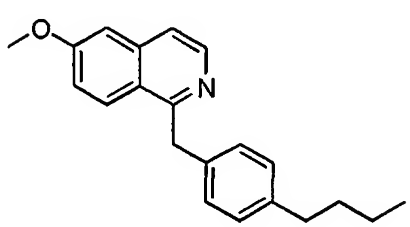


実施例Ｂ１４４の化合物1.0g（5.2ミリモル）のベンゼン（10ml）溶液に、塩化チオニル0.76ml（10ミリモル）を加え、還流下２時間撹拌した。濃縮後、さらにベンゼンと共沸させることにより過剰の塩化チオニルを除去した。得られた残渣と実施例Ｂ１４３の化合物0.87g（5.2ミリモル）のエーテル（5ml）溶液に、水酸化ナトリウム水溶液（水酸化ナトリウム0.21gを水4.2mlに溶解した。）を加え室温で３０分間激しく撹拌した。エーテル層を分離後、減圧濃縮し、表題化合物600mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.94(3H, t), 1.31-1.40(2H, m), 1.57-1.63(2H, m), 2.60(2H, m), 3.30-3.37(1H, m), 3.56(2H, s), 3.60-3.66(1H, m), 3.80(3H, s), 3.81(1H, d), 4.79-4.81(1H, m), 6.80-6.89(3H, m), 7.10(2H, d), 7.16(2H, d), 7.20-7.25(1H, m)

実施例Ｂ１４６

1-(4-ブチルベンジル)-6-メトキシイソキノリン



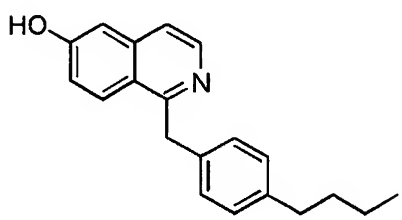
実施例Ｂ１４５の化合物600mg（1.7ミリモル）のアセトニトリル（15ml）溶液に、オキシ塩化リン1.6mlを加え、還流下１時間３０分間撹拌し

た。氷冷後、５％炭酸水素ナトリウム水溶液を用いてアルカリ性にした後、酢酸エチルで抽出し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物82mg を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.53(2H, t), 3.92(3H, s), 4.57(2H, s), 7.05-7.07(3H, m), 7.13-7.18(3H, m), 7.45(1H, d), 8.06(1H, d), 8.41(1H, d)

実施例Ｂ１４７

1-(4-ブチルベンジル)-6-イソキノリノール

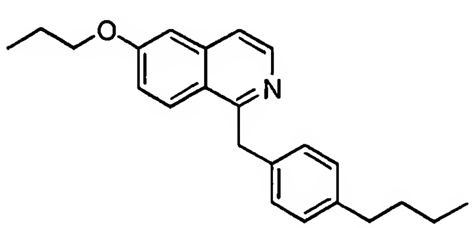


実施例Ｂ１４６の化合物82mgに４７％臭化水素水を加え、還流下１９時間攪拌した。減圧濃縮した後、水を加え、炭酸ナトリウムで中和することにより結晶を析出させた。得られた結晶をろ取し、水で洗浄後、結晶を乾燥し、表題化合物74mg を得た。

^{1}H-NMR(CD_3OD)δ(ppm):0.89(3H, t), 1.25-1.34(2H, m), 1.49-1.57(2H, m), 2.52(2H, t), 4.63(2H, s), 7.03-7.13(6H, m), 7.49(1H, d), 8.10(1H, d), 8.18(1H, d)

実施例Ｂ１４８

1-(4-ブチルベンジル)-6-プロポキシイソキノリン

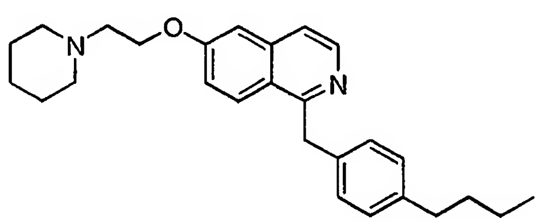


実施例Ｂ１４７の化合物20mg(0.069ミリモル)と1-ヨードプロパン0.4ml(4.1ミリモル)のトルエン(1.0ml)溶液に炭酸銀40mg(0.14ミリモル)を加え、遮光下５０℃で４時間撹拌した。室温まで冷却後、セライトを用いて濾過し、トルエン－メタノール(9:1)混合溶液で洗浄した。得られた濾液を減圧濃縮した後、残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物13mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.08(3H, t), 1.30-1.33(2H, m), 1.51-1.57(2H, m), 1.86-1.91(2H, m), 2.54(2H, t), 4.05(2H, t), 4.58(2H, s), 7.05-7.07(3H, m), 7.14-7.18(3H, m), 7.43-7.44(1H, m), 8.05-8.07(1H, m), 8.40-8.41(1H, m)

実施例Ｂ１４９

1-(4-ブチルベンジル)-6-(2-ピペリジノエトキシ)イソキノリン

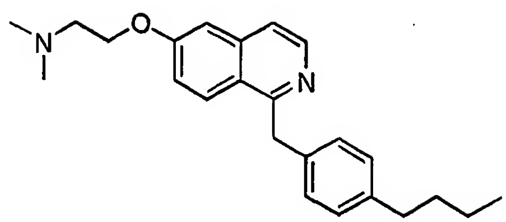


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.46-1.57(8H, m), 2.50-2.54(6H, m), 2.83-2.86(2H, m), 4.23(2H, t), 4.56(2H, s), 7.04-7.06(3H, m), 7.13-7.17(3H, m), 7.43(1H, d), 8.04(1H, d), 8.40(1H, d)

実施例Ｂ１５０

N-(-{[1-(4-ブチルベンジル)-6-イソキノリル]オキシ}エチル)-N,N-ジメチルアミン

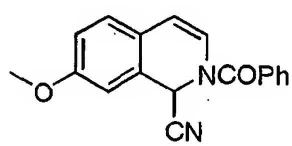


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.49-1.57(2H, m), 2.37(6H, s), 2.52(2H, t), 2.80(2H, t), 4.19(2H, t), 4.57(2H, s), 7.04-7.06(3H, m), 7.15-7.19(3H, m), 7.43(1H, d), 8.05(1H, d), 8.40(1H, d)

実施例Ｂ１５１

2-ベンゾイル-7-メトキシ-1,2-ジヒドロ-1-イソキノリンカルボニトリル

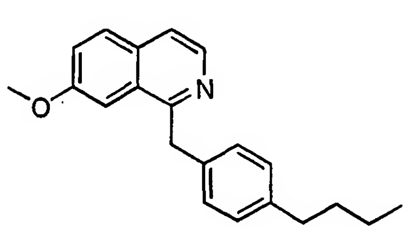


Tetrahedron, 27, 1253 (1971)に基づいて合成した7-メトキシイソキノリンを実施例Ｂ１４０と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.87(3H, s), 6.03(1H, brd), 6.56-6.54(2H, m), 6.90(1H, s), 6.95(1H, dd), 7.17(1H, d), 7.46-7.50(2H, m), 7.54-7.62(3H, m)

実施例Ｂ１５２

1-(4-ブチルベンジル)-7-メトキシイソキノリン

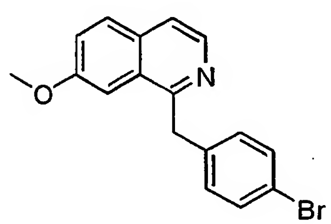


実施例Ｂ１の化合物と実施例Ｂ１５１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.56-1.58(2H, m), 2.55(2H, t), 3.82(3H, s), 4.59(2H, s), 7.07(2H, d), 7.20(2H, d), 7.26-7.29(1H, m), 7.35(1H, d), 7.49(1H, d), 7.70(1H, d), 8.38-8.40(1H, m)

実施例Ｂ１５３

1-(4-ブロモベンジル)-7-メトキシイソキノリン

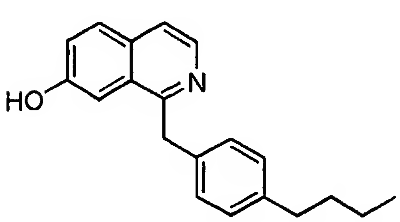


実施例Ｂ３１の化合物と実施例Ｂ１５１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.84(3H, s), 4.57(2H, s), 7.14-7.16(2H, m), 7.26(1H, s), 7.29-7.32(1H, m), 7.37-7.39(2H, m), 7.51(1H, d), 7.73(1H, d), 8.39(1H, d)

実施例Ｂ１５４

1-(4-ブチルベンジル)-7-イソキノリノール



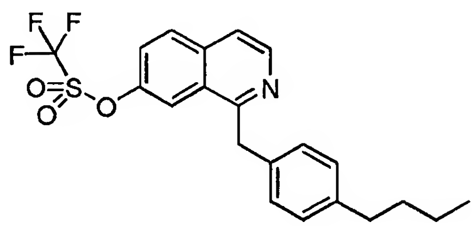
実施例Ｂ１５２の化合物を実施例Ｂ１４７と同様にして表題化合物を得た。

^{1}H-NMR(DMSO-d_6)δ(ppm):0.83(3H, t), 1.21-1.26(2H, m), 1.44-1.4

8(2H, m), 4.68(2H, s), 7.11(2H, d), 7.18(2H, d), 7.59-7.62(2H, m), 8.10-8.17(2H, m), 8.38(1H, d), 10.9(1H, brs) （但し、ブチル基のメチレンプロトン２個分がDMSOのシグナルに重なっていて見えない。）

実施例Ｂ１５５

1-(4-ブチルベンジル)-7-イソキノリル トリフルオロメタンスルフォネート

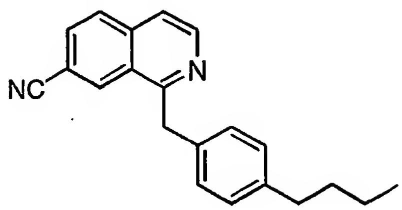


実施例Ｂ１５４の化合物1.0g（2.7ミリモル）のジメチルホルムアミド（30ml）溶液にJ. Org. Chem.,64, 7638(1999)に基づいて合成した4-ニトロフェノールトリフラート0.72g（2.7ミリモル）と炭酸カリウム1.1g（8.1ミリモル）を加え、室温で２時間攪拌した。水を加え、酢酸エチルで抽出した。酢酸エチル層を１規定水酸化ナトリウムと飽和食塩水で洗浄し、硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物1.0gを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.27-1.37(2H, m), 1.51-1.59(2H, m), 2.54(2H, t), 5.10(2H, s), 6.38(1H, s), 6.95(2H, d), 7.04(2H, d), 7.44(1H, d), 7.55(1H, d), 7.75(1H, d), 8.45(1H, d)

実施例Ｂ１５６

1-(4-ブチルベンジル)-7-イソキノリンカルボニトリル

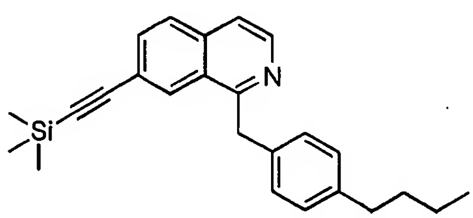


窒素雰囲気下、実施例Ｂ１５５の化合物400mg（0.95ミリモル）のジメチルホルムアミド（2ml）溶液に、シアン化亜鉛215mg（1.8ミリモル）、テトラキストリフェニルフォスフィンパラジウム41mg（0.035ミリモル）そして塩化リチウム120mg（2.8ミリモル）を加え１２０℃で２時間攪拌した。室温まで冷却後、飽和炭酸水素ナトリウムを加え、酢酸エチルで抽出した。酢酸エチル層を飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物71mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.26-1.35(2H, m), 1.47-1.55(2H, m), 2.50(2H, t), 4.91(2H, s), 6.97(2H, d), 7.07(2H, d), 7.28-7.31(1H, m), 7.42(1H, d), 7.51(1H, d), 7.74(1H, d), 8.34(1H, d)

実施例Ｂ１５７

1-(4-ブチルベンジル)-7-[2-(1,1,1-トリメチルシリル)-1-エチニル]イソキノリン



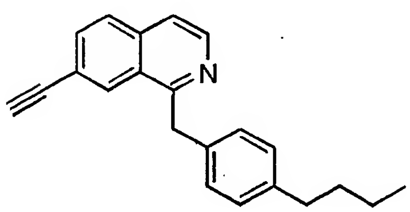
実施例Ｂ１５５の化合物100mg（0.24ミリモル）とトリメチルシリルアセチレン65μl（0.47ミリモル）のジメチルホルムアミド（3.0ml）溶液に、酢酸パラジウム11mg（0.047ミリモル）、1,1-ビスジフェニルフォス

フィノフェロセン72mg（0.13ミリモル）そして塩化リチウム25mg（0.59ミリモル）を加え窒素置換した。この溶液にトリエチルアミン59μl（0.43ミリモル）とヨウ化銅2mg（0.018ミリモル）を加え、８０℃で２１時間撹拌した。室温まで冷却後、水と酢酸エチルを加え分配した。酢酸エチル層を水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物7.0mg を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.28-0.32(9H, m), 0.92(3H, t), 1.32-1.38(2H, m), 1.54-1.57(2H, m), 2.57(2H, t), 4.63(2H, s), 7.10(2H, d), 7.20(2H, d), 7.52(1H, d), 7.67-7.69(1H, m), 7.75(1H, d), 8.34(1H, d), 8.51(1H, d)

実施例Ｂ１５８

1-(4-ブチルベンジル)-7-(1-エチニル)イソキノリン

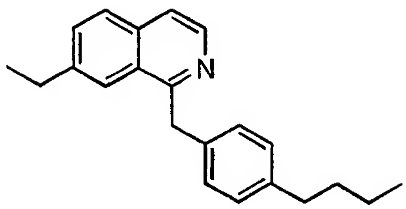


実施例Ｂ１５７の化合物6mg（0.016ミリモル）のメタノール(1.0ml)溶液に、炭酸カリウム13mg（0.094ミリモル）を加え室温で１時間撹拌した。減圧濃縮した後、得られた残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物3.0mg を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.91(3H, t), 1.29-1.38(2H, m), 1.52-1.57(2H, m), 2.55(2H, t), 3.19(1H, s), 4.62(2H, s), 7.09(2H, d), 7.20(2H, d), 7.53(1H, d), 7.67-7.69(1H, m), 7.77(1H, d), 8.36(1H, s), 8.52(1H, d)

実施例Ｂ１５９

1-(4-ブチルベンジル)-7-エチルイソキノリン

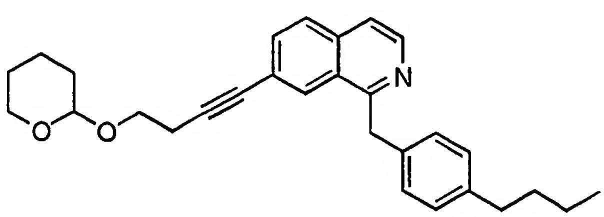


実施例B158の化合物2.0mgのテトラヒドロフラン(2.0ml)溶液に、パラジウム－炭素5.0mg(10%)を加え、室温で窒素雰囲気下(1atom)1時間撹拌した。触媒を濾去し、濾液を濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物0.21mgを得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(6H, t), 1.25-1.32(2H, m), 1.48-1.57(2H, m), 2.53(2H, t), 2.80(2H, q), 4.62(2H, s), 7.06(2H, d), 7.20(2H, d), 7.49-7.52(2H, m), 7.73(1H, d), 7.95(1H, s), 8.43(1H, d)

実施例B160

1-(4-ブチルベンジル)-7-[4-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ブチニル]イソキノリン



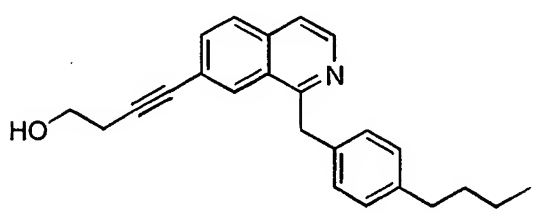
実施例B155の化合物100mg(0.24ミリモル)と2-(3-ブチニルオキシ)テトラヒドロ-2H-ピラン73mg(0.47ミリモル)のジメチルホルムアミド(3.0ml)溶液に、酢酸パラジウム11mg(0.047ミリモル)、1,1-ビスジフェニルフォスフィノフェロセン72mg(0.13ミリモル)そして塩化リチウム25mg(0.59ミリモル)を加え系内を窒素置換した。さらに、トリエチルアミン59μl(0.43ミリモル)とヨウ化銅2mg(0.018ミリモル)を

加え、80℃で24時間攪拌した。室温まで冷却後、水を加え酢酸エチルで抽出した。酢酸エチル層を水で洗浄し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物25mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.28-1.38(2H, m), 1.52-1.67(6H, m), 1.72-1.79(1H, m), 1.79-1.88(1H, m), 2.54(2H, t), 2.78(2H, t), 3.53-3.56(1H, m), 3.66-3.72(1H,m), 3.91-3.99(2H, m), 4.60(2H, s), 4.71-4.73(1H, m), 7.08(2H, d), 7.19(2H, d), 7.50(1H, d), 7.59-7.62(1H, m), 7.72(1H, d), 8.24(1H, s), 8.48(1H, d)

実施例B161

4-[1-(4-ブチルベンジル)-7-イソキノリル]-3-ブチン-1-オール

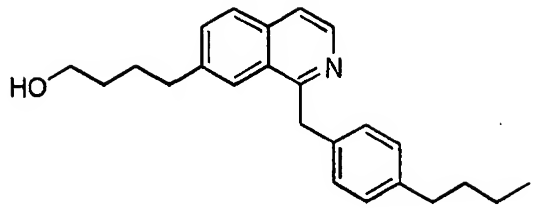


実施例B160の化合物を実施例B29と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.39(2H, m), 1.51-1.57(2H, m), 1.83(1H, brs), 2.55(2H, t), 2.75(2H, t), 3.84-3.89(2H, m), 4.60(2H, s), 7.08(2H, d), 7.18(2H, d), 7.50(1H, d), 7.60-7.62(1H, m), 7.73(1H, d), 8.25(1H, s), 8.48(1H, d)

実施例B162

4-[1-(4-ブチルベンジル)-7-イソキノリル]-1-ブタノール

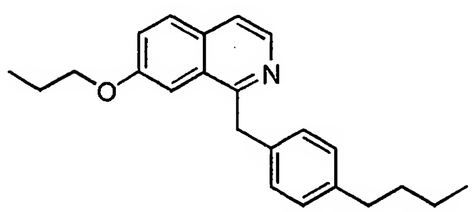


実施例Ｂ１６１の化合物を実施例Ｂ３０と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.28-1.36(2H, m), 1.50-1.59(4H, m), 1.67-1.77(3H, m), 2.53(2H, t), 2.79(2H, t), 3.63(2H, t), 4.62(2H, s), 7.06(2H, d), 7.18(2H, d), 7.47-7.52(2H, m), 7.73(1H, d), 7.92(1H, s), 8.43(1H, d)

実施例Ｂ１６３

1-(4-ブチルベンジル)-7-プロポキシイソキノリン

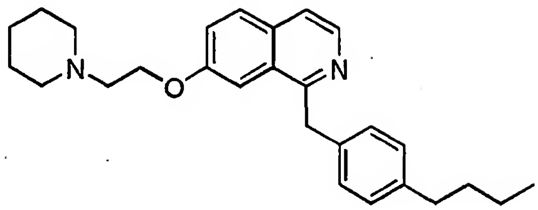


実施例Ｂ１５４の化合物を実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.05(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 1.76-1.84(2H, m), 2.53(2H, t), 3.92(2H, t), 4.58(2H, s), 7.06(2H, d), 7.19(2H, d), 7.26-7.29(1H, m), 7.34(1H, d), 7.48(1H, d), 7.70(1H, d), 8.38(1H, d)

実施例Ｂ１６４

1-(4-ブチルベンジル)-7-(2-ピペリジノエトキシ)イソキノリン

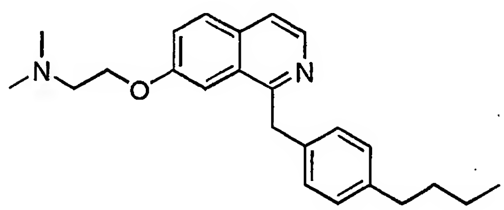


実施例B148と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.43-1.58(4H, m), 1.61-1.69(4H, m), 2.51-2.55(6H, m), 2.79(2H, t), 4.11(2H, t), 4.57(2H, s), 7.06(2H, d), 7.18(2H, d), 7.28-7.30(1H, m), 7.36(1H, d), 7.48(1H, d), 7.70(1H, d), 8.38(1H, d)

実施例B165

N-(2-{[1-(4-ブチルベンジル)-7-イソキノリル]オキシ}エチル)-*N*,*N*-ジメチルアミン

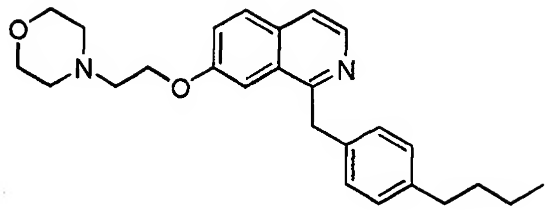


実施例B148と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.57(2H, m), 2.35(6H, s), 2.53(2H, t), 2.75(2H, t), 4.06(2H, t), 4.58(2H, s), 7.06(2H, d), 7.18(2H, d), 7.30-7.33(1H, m), 7.36(1H, d), 7.48(1H, d), 7.70(1H, d), 8.39(1H, d)

実施例B166

1-(4-ブチルベンジル)-7-イソキノリル(2-モルフォリノエチル)エーテル

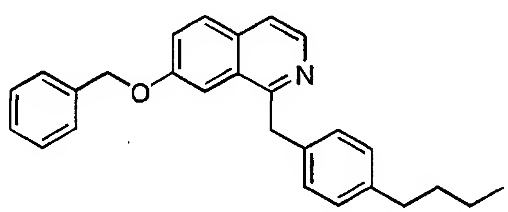


実施例B148と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.51-2.58(6H, m), 2.81(2H, t), 3.75(4H, t), 4.11(2H, t), 4.58(2H, s), 7.06(2H, d), 7.17(2H, d), 7.28-7.31(1H, m), 7.35(1H, d), 7.49(1H, d), 7.71(1H, d), 8.39(1H, d)

実施例B167

7-(ベンジルオキシ)-1-(4-ブチルベンジル)イソキノリン

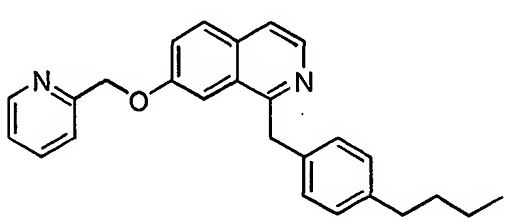


実施例B148と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.54(2H, m), 2.54(2H, t), 4.54(2H, s), 5.06(2H, s), 7.05(2H, d), 7.14(2H, d), 7.34-7.43(7H, m), 7.49(1H, d), 7.72(1H, d), 8.39(1H, d)

実施例B168

1-(4-ブチルベンジル)-7-(2-ピリジルメトキシ)イソキノリン

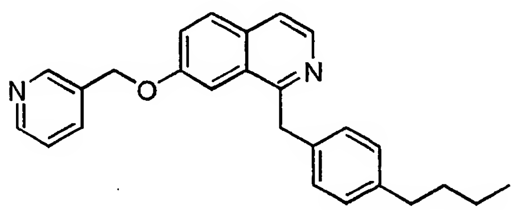


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.49-1.57(2H, m), 2.52(2H, t), 4.51(2H, s), 5.25(2H, s), 7.02(2H, d), 7.14(2H, d), 7.24-7.27(1H, m), 7.40(1H, dd), 7.47-7.50(3H, m), 7.68-7.72(1H, d), 7.74(1H, d), 8.39(1H, d), 8.64-8.66(1H, m)

実施例Ｂ１６９

1-(4-ブチルベンジル)-7-(3-ピリジルメトキシ)イソキノリン

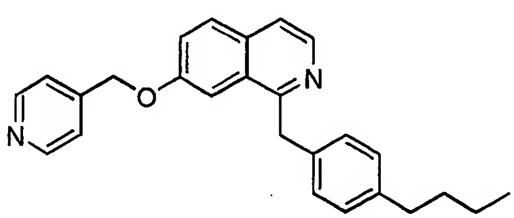


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.54(2H, t), 4.57(2H, s), 5.06(2H, s), 7.07(2H, d), 7.15(2H, d), 7.31-7.36(2H, m), 7.42(1H, d), 7.51(1H, d), 7.74-7.76(2H, m), 8.42(1H, d), 8.61-8.62(1H, m), 8.69-8.70(1H, m)

実施例Ｂ１７０

1-(4-ブチルベンジル)-7-(4-ピリジルメトキシ)イソキノリン



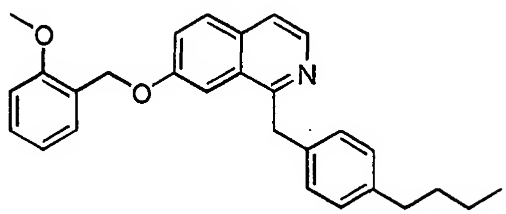
実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 2.54(2H, t), 4.53(2H, s), 5.09(2H, s), 7.04(2H, d), 7.09(2H, d), 7.33-7.39(4H, m), 7.51(1H, d), 7.76(1H, d), 8.41(1H,

d), 8.63-8.64(2H, m)

実施例B171

1-(4-ブチルベンジル)-7-[(2-メトキシベンジル)オキシ]イソキノリン

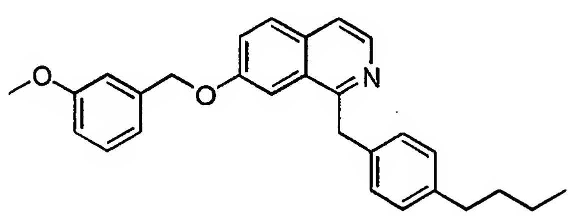


実施例B148と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.57(2H, m), 2.53(2H, t), 3.82(3H, s), 4.52(2H, s), 5.04(2H, s), 6.88-6.91(1H, m), 6.99-7.02(2H, m), 7.05(2H, d), 7.14(2H, d), 7.32(1H, t), 7.36(1H, dd), 7.43(1H, d), 7.48(1H, d), 7.72(1H, d), 8.39(1H, d)

実施例B172

1-(4-ブチルベンジル)-7-[(3-メトキシベンジル)オキシ]イソキノリン

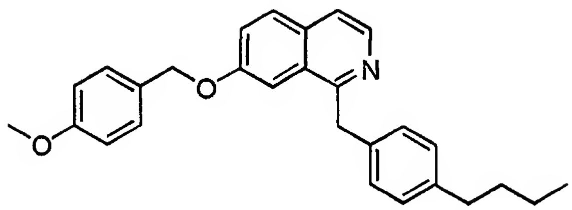


実施例B148と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 2.53(2H, t), 3.90(3H, s), 4.53(2H, s), 5.16(2H, s), 6.93-6.98(2H, m), 7.03(2H, d), 7.15(2H, d), 7.30-7.35(1H, m), 7.37(1H, dd), 7.41-7.43(1H, m), 7.47(1H, d), 7.51(1H, d), 7.71(1H, d), 8.37(1H, d)

実施例B173

1-(4-ブチルベンジル)-7-[(4-メトキシベンジル)オキシ]イソキノリン

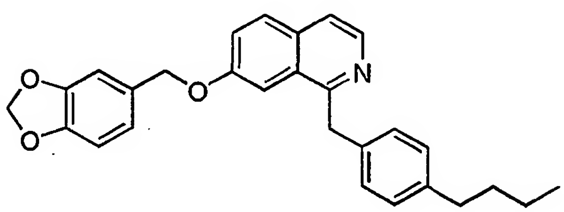


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 1.27-1.37(2H, m), 1.51-1.57(2H, m), 2.54(2H, t), 3.83(3H, s), 4.55(2H, s), 4.99(2H, s), 6.93(2H, d), 7.06(2H, d), 7.15(2H, d), 7.32-7.36(3H, m), 7.44(1H, d), 7.48(1H, d), 7.71(1H, d), 8.38(1H, d)

実施例Ｂ１７４

7-(1,3-ベンゾオキシオール-5-イルメトキシ)-1-(4-ブチルベンジル)イソキノリン

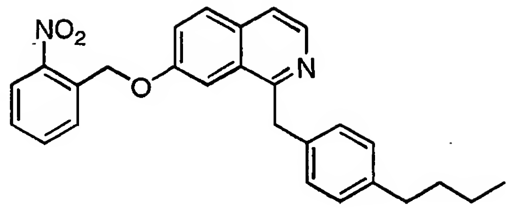


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 1.27-1.37(2H, m), 1.51-1.57(2H, m), 2.54(2H, t), 4.55(2H, s), 4.95(2H, s), 5.98(2H, s), 6.82(1H, d), 6.88(1H, dd), 6.92(1H, d), 7.06(2H, d), 7.15(2H, d), 7.33(1H, dd), 7.42(1H, d), 7.48(1H, d), 7.72(1H, d), 8.39(1H, d)

実施例Ｂ１７５

1-(4-ブチルベンジル)-7-[(2-ニトロベンジル)オキシ]イソキノリン

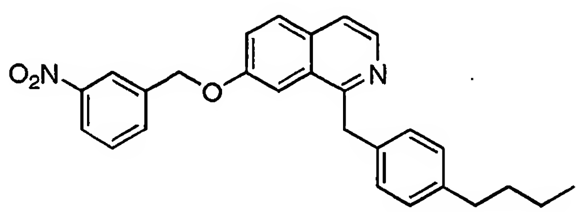


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.87(3H, t), 1.26-1.34(2H, m), 1.48-1.56(2H, m), 2.51(2H, t), 4.53(2H, s), 5.49(2H, s), 7.03(2H, d), 7.14(2H, d), 7.40(1H, dd), 7.430-7.434(1H, m), 7.45-7.49(1H, m), 7.51(1H, d), 7.64-7.68(1H, m), 7.76(1H, d), 7.85-7.87(1H, m), 8.22-8.24(1H, d), 8.41(1H, d)

実施例Ｂ１７６

1-(4-ブチルベンジル)-7-[(3-ニトロベンジル)オキシ]イソキノリン

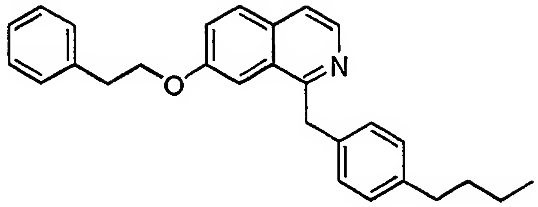


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 2.54(2H, t), 4.55(2H, s), 5.14(2H, s), 7.05(2H, d), 7.11(2H, d), 7.37-7.40(2H, m), 7.51(1H, d), 7.55-7.59(1H, m), 7.73-7.78(2H, m), 8.19-8.22(1H, m), 8.32-8.33(1H, m), 8.42(1H, d)

実施例Ｂ１７７

1-(4-ブチルベンジル)-7-(フェネチルオキシ)イソキノリン

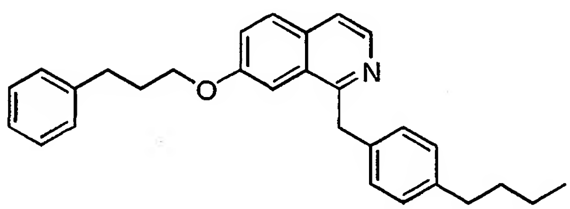


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.49-1.57(2H, m), 2.52(2H, t), 3.10(2H, t), 4.18(2H, t), 4.56(2H, s), 7.04(2H, d), 7.16(2H, d), 7.26-7.28(4H, m), 7.33-7.35(3H, m), 7.48(1H, d), 7.70(1H, d), 8.38-8.39(1H, m)

実施例Ｂ１７８

1-(4-ブチルベンジル)-7-(3-フェニルプロポキシ)イソキノリン

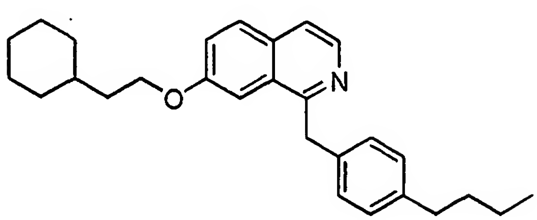


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.49-1.57(2H, m), 2.09-2.15(2H, m), 2.52(2H, t), 2.82(2H, t), 3.97(2H, t), 4.55(2H, s), 7.04(2H, d), 7.16(2H, d), 7.20-7.23(3H, m), 7.27-7.33(4H, m), 7.48(1H, d), 7.70(1H, d), 8.38(1H, d)

実施例Ｂ１７９

1-(4-ブチルベンジル)-7-(2-シクロヘキシルエトキシ)イソキノリン

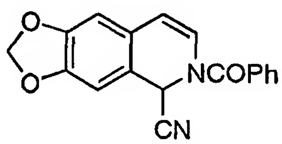


実施例Ｂ１４８と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.89(3H, t), 0.94-1.02(2H, m), 1.17-1.36(4H, m), 1.36-1.57(4H, m), 1.65-1.76(7H, m), 2.53(2H, t), 3.98(2H, t), 4.58(2H, s), 7.06(2H, d), 7.19(2H, d), 7.25-7.28(1H, m), 7.33(1H, d), 7.47(1H, d), 7.69(1H, d), 8.37(1H, d)

実施例Ｂ１８０

6-ベンゾイル-5,6-ジヒドロ[1,3]ジオキソロ[4,5-g]イソキノリン-5-カルボニトリル

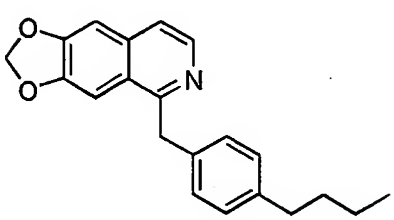


[1,3]ジオキソロ[4,5-g]イソキノリンを実施例Ｂ１４０と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):5.94-5.96(1H, m), 6.03(1H, d), 6.04(1H, d), 6.47-6.54(2H, m), 6.70(1H, s), 6.83(1H, s), 7.45-7.49(2H, m), 7.54-7.62(3H, m)

実施例Ｂ１８１

5-(4-ブチルベンジル)[1,3]ジオキソロ[4,5-g]イソキノリン



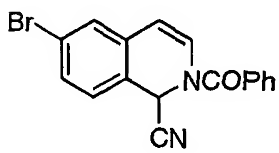
実施例Ｂ１８０の化合物と実施例Ｂ１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.57(2H, m), 2.54(2H, t), 4.50(2H, s), 6.05(2H, s), 7.05-7.07(3H,

m), 7.16(2H, d), 7.38(7.40(2H, m), 8.35(1H, d)

実施例Ｂ１８２

2-ベンゾイル-6-ブロモ-1,2-ジヒドロ-1-イソキノリンカルボニトリル

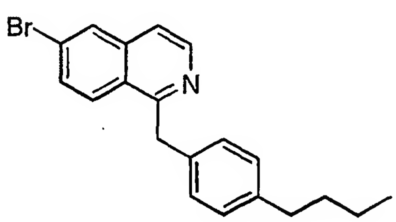


J. Am. Chem. Soc., 183(1942)に基づいて合成した6-ブロモイソキノリンを実施例Ｂ１４０と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):6.01(1H, d), 6.53(1H, brs), 6.70(1H, brd), 7.24(1H, d), 7.33(1H, d), 7.47-7.51(3H, m), 7.56(3H, m)

実施例Ｂ１８３

6-ブロモ-1-(4-ブチルベンジル)イソキノリン

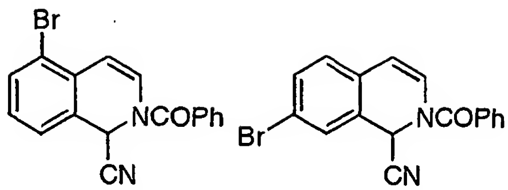


実施例Ｂ１８２の化合物と実施例Ｂ１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.53(2H, t), 4.60(2H, s), 7.06(2H, d), 7.15(2H, d), 7.46(1H, d), 7.59(1H, q), 7.98(1H, d), 8.02(1H, d), 8.51(1H, d)

実施例Ｂ１８４

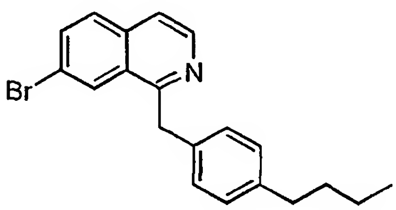
2-ベンゾイル-5-ブロモ-1,2-ジヒドロ-1-イソキノリンカルボニトリルと2-ベンゾイル-7-ブロモ-1,2-ジヒドロ-1-イソキノリンカルボニトリルの混合物



J. Am. Chem. Soc., 61, 183(1939)に基づいて合成した5-または7-ブロモイソキノリンを実施例Ｂ１４０と同様にして表題化合物を得た。得られた化合物は分離精製することなく次の反応に用いた。

実施例Ｂ１８５

7-ブロモ-1-(4-ブチルベンジル)イソキノリン

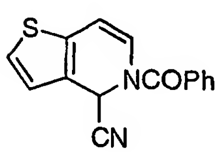


実施例Ｂ１８４の化合物と実施例Ｂ１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.58(2H, m), 2.55(2H, t), 4.58(2H, s), 7.09(2H, d), 7.18(2H, d), 7.51-7.53(1H, m), 7.69-7.70(2H, m), 8.33-8.34(1H, m), 8.52(1H, d)

実施例Ｂ１８６

5-ベンゾイル-4,5-ジヒドロチエノ[3,2-c]ピリジン-4-カルボニトリル

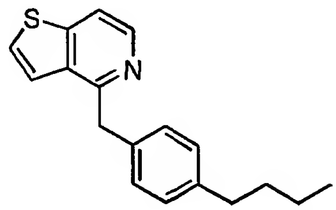


J. Heterocycl. Chem., 30, 183 (1993)に基づいて合成したチエノ[3,2-c]ピリジンを実施例Ｂ１４０と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):6.05(1H, d), 6.57(1H, brd), 6.66(1H, s), 7.07(1H, d), 7.32(1H, d), 7.46-7.50(2H, m), 7.54-7.62(3H, m)

実施例Ｂ１８７

4-(4-ブチルベンジル)チエノ[3,2-c]ピリジン

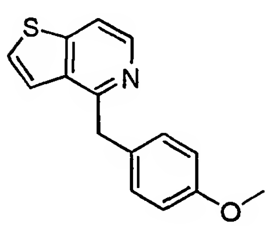


実施例Ｂ１８６の化合物と実施例Ｂ１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):0.90(3H, t), 1.27-1.37(2H, m), 1.51-1.59(2H, m), 2.54(2H, t), 4.47(2H, s), 7.07(2H, d), 7.19(2H, d), 7.42(1H, d), 7.47(1H, dd), 7.68(1H, d), 8.41(1H, d)

実施例Ｂ１８８

4-(4-メトキシベンジル)チエノ[3,2-c]ピリジン

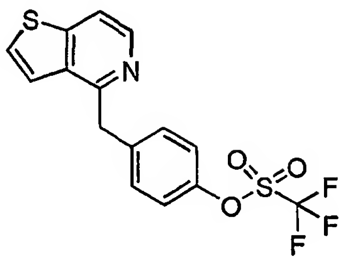


実施例Ｂ１８６の化合物と4-メトキシベンジルクロリドを実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):3.75(3H, s), 4.44(2H, s), 6.79-6.82(2H, m), 7.19-7.22(2H, m), 7.43(1H, d), 7.46(1H, dd), 7.68(1H, d), 8.41(1H, d)

実施例Ｂ１８９

4-(チエノ[3,2-c]ピリジン-4-イルメチル)フェニル トリフルオロメタンスルフォネート

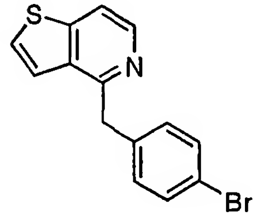


0℃に冷却した実施例B188の化合物510mg（2.0ミリモル）の塩化メチレン（10ml）溶液に、三臭化ホウ素の塩化メチレン溶液10ml（1.0M, 10ミリモル）を滴下し、その温度で1時間半撹拌した。飽和炭酸水素ナトリウム水溶液を加え弱アルカリ性にした後、酢酸エチルで抽出し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。得られた残渣をピリジンに溶解し、0℃に冷却した後、トリフルオロメタンスルフォニックアンハイドライド0.34ml（2.1ミリモル）を滴下し、その温度で2時間撹拌した。氷水に注ぎ、酢酸エチルで抽出し、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残渣をシリカゲルクロマトグラフィーで精製し、表題化合物312mg を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.52(2H, s), 7.16-7.18(2H, m), 7.36(2H, m), 7.43-7.44(1H, m), 7.49(1H, d), 7.73(1H, d), 8.42(1H, d)

実施例B190

4-(4-ブロモベンジル)チエノ[3,2-c]ピリジン



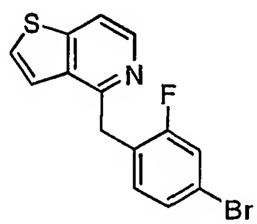
実施例B186の化合物と実施例B31の化合物を実施例B2と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.45(2H, s), 7.14-7.16(2H, m), 7.37-7.39(2H, m), 7.41-7.43(1H, m), 7.45(1H, d), 7.71(1H, d), 8.41(1H,

d)

実施例Ｂ１９１

4-(4-ブロモ-2-フルオロベンジル)チエノ[3,2-c]ピリジン

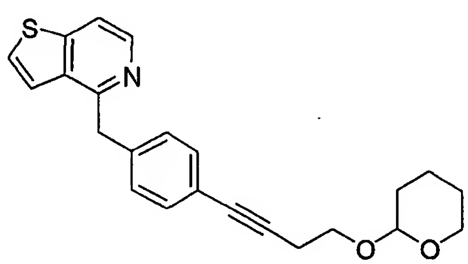


実施例Ｂ１８６の化合物と4-ブロモ2-フルオロベンジブロミドを実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.46(2H, s), 7.11(1H, t), 7.15-7.18(1H, m), 7.22-7.25(1H, m), 7.47(1H, d), 7.49(1H, d), 7.71(1H, d), 8.41(1H, d)

実施例Ｂ１９２

4-{4-[4-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ブチニル]ベンジル}チエノ[3,2-c]ピリジン

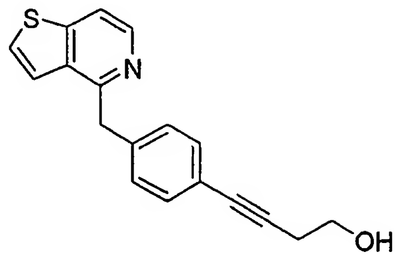


実施例Ｂ１８９の化合物と2-(3-ブチニルオキシ)テトラヒドロ-2H-ピランを実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.40-1.90(6H, m), 2.69(2H, t), 3.45-3.65(2H, m), 3.78-3.95(2H, m), 4.48(2H, s), 4.66-4.69(1H, m), 7.18(2H, d), 7.27(2H, d), 7.41(1H, d), 7.44(1H, d), 7.70(1H, d), 8.41(1H, d).

実施例Ｂ１９３

4-[4-(チエノ[3,2-c]ピリジン-4-イルメチル)フェニル]-3-ブチン-1-オール



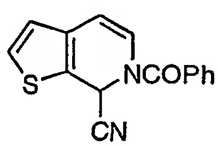
実施例Ｂ１９２の化合物を実施例Ｂ４７と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.67(2H, t), 3.79(2H, t), 4.50(2H, s), 7.20(2H, d), 7.32(2H, d), 7.41(1H, d), 7.44(1H, d), 7.71(1H, d), 8.42(1H, d).

水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ１９４

6-ベンゾイル-6,7-ジヒドロチエノ[2,3-c]ピリジン-7-カルボニトリル

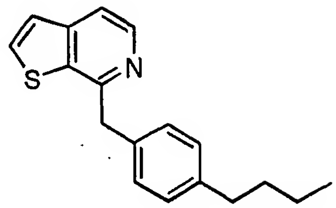


J. Heterocycl. Chem., 30, 183(1993)に基づいて合成したチエノ[2,3-c]ピリジンを実施例Ｂ１４０と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):6.07(1H, d), 6.56(1H, brd), 6.75(1H, s), 6.97(1H, d), 7.37(1H, d), 7.46-7.51(2H, m), 7.54-7.64(3H, m)

実施例Ｂ１９５

7-(4-ブチルベンジル)チエノ[2,3-c]ピリジン

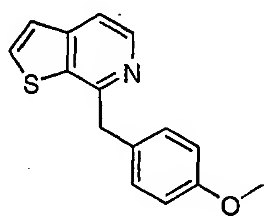


実施例Ｂ１９４の化合物と実施例Ｂ１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.59(2H, m), 2.55(2H, t), 4.40(2H, s), 7.09(2H, d), 7.28(2H, d), 7.34(1H, d), 7.57(1H, d), 7.62(1H, d), 8.47(1H, d)

実施例Ｂ１９６

7-(4-メトキシベンジル)チエノ[2,3-c]ピリジン

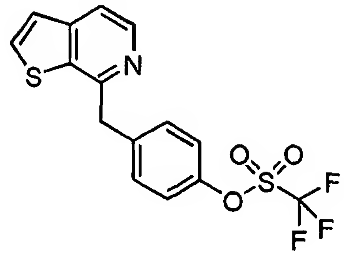


実施例Ｂ１９４の化合物と4-メトキシベンジルクロリドを実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm):3.76(3H, s), 4.38(2H, s), 6.81-6.83(2H, m), 7.28-7.30(2H, m), 7.35(1H, d), 7.57(1H, d), 7.62(1H, d), 8.47(1H, d)

実施例Ｂ１９７

4-(チエノ[2,3-c]ピリジン-7-イルメチル)フェニル トリフルオロメタンスルフォネート

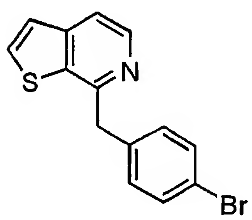


実施例Ｂ１９６の化合物を実施例Ｂ１８９と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.44(2H, s), 7.17-7.19(2H, m), 7.38-7.40(1H, m), 7.44-7.46(2H, m), 7.61(1H, d), 7.65-7.67(1H, m), 8.47-8.49(1H, m)

実施例Ｂ１９８

7-(4-ブロモベンジル)チエノ[2,3-c]ピリジン

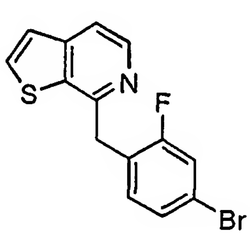


実施例Ｂ１９４の化合物と実施例Ｂ３１の化合物を実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.37(2H, s), 7.23-7.25(2H, m), 7.37(1H, d), 7.39-7.41(2H, m), 7.59(1H, d), 7.63-7.65(1H, m), 8.47(1H, d)

実施例Ｂ１９９

7-(4-ブロモ-2-フルオロベンジル)チエノ[2,3-c]ピリジン

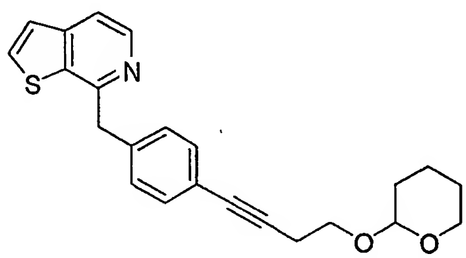


実施例Ｂ１９４の化合物と4-ブロモ2-フルオロベンジブロミドを実施例Ｂ２と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.40-4.41(2H, m), 7.12-7.20(2H, m), 7.23-7.26(1H, m), 7.37-7.39(1H, m), 7.59-7.62(1H, m), 7.65-7.67(1H, m), 8.45-8.47(1H, m)

実施例Ｂ２００

7-{4-[4-(テトラヒドロ-2H-2-ピラニルオキシ)-1-ブチニル]ベンジル}チエノ[2,3-c]ピリジン

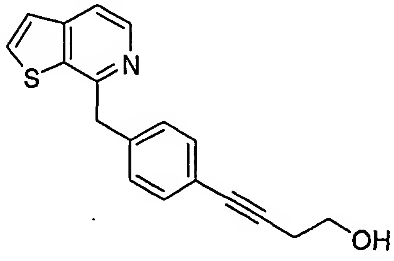


実施例Ｂ１９７の化合物と2-(3-ブチニルオキシ)テトラヒドロ-2H-ピランを実施例Ｂ４２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.50-1.90(6H, m), 2.69(2H, t), 3.49-3.54(1H, m), 3.58-3.65(1H, m), 3.85-3.95(2H, m), 4.41(2H, s), 4.68(1H, t), 7.26-7.31(4H, m), 7.36(1H, d), 7.58(1H, d), 7.63(1H, d), 8.47(1H, d).

実施例Ｂ２０１

4-[4-(チエノ[2,3-c]ピリジン-7-イルメチル)フェニル]-3-ブチン-1-オール



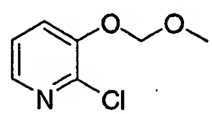
実施例Ｂ２００の化合物を実施例Ｂ４７と同様に処理し、表題化合物

を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):1.99(1H, brs), 2.67(2H, t), 3.79(2H, t), 4.42(2H, s), 7.27-7.34(4H, m), 7.36(1H, d), 7.59(1H, d), 7.64(1H, d), 8.47(1H, d).

実施例B202

2-クロロ-3-(メトキシメトキシ)ピリジン

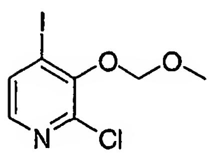


窒素雰囲気下、氷冷した2-クロロ-3-ヒドロキシピリジン2.05g (15.8ミリモル)のテトラヒドロフラン(30ml)溶液に、66%水素化ナトリウム633mg (17.4ミリモル)を加え、その温度で15分間攪拌した。その反応溶液にクロロメチルメチルエーテル1.32ml (17.4ミリモル)を加え、その温度で30分間攪拌後、さらに室温で2時間攪拌した。水を加え、酢酸エチルを用いて抽出し、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物2.44gを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 3.53(3H, s), 5.28(2H, s), 7.19(1H, dd), 7.49(1H, dd), 8.06(1H, dd)

実施例B203

2-クロロ-4-ヨード-3-(メトキシメトキシ)ピリジン



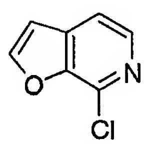
窒素雰囲気下、-78℃に冷却した1.51M t-ブチルリチウム-n-ペンタン溶液8.01ml (12.1ミリモル)のジエチルエーテル(15ml)溶液に、実施例B202の化合物1.40g (8.06ミリモル)のジエチルエーテル8ml溶液を滴下し、その温度で15分間攪拌した。その反応溶液にヨウ素3.07g (12.

1ミリモル)を加え、徐々に室温まで昇温させた。チオ硫酸ナトリウム水溶液を加え、ジエチルエーテル層を分配し、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物356mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 3.73(3H, s), 5.22(2H, s), 7.69(1H, d), 7.80(1H, d)

実施例B204

7-クロロフロ[2,3-c]ピリジン

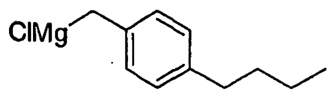


実施例B203の化合物36.6mg (0.143ミリモル)、テトラキストリフェニルホスフィンパラジウム16.5mg (0.0143ミリモル)そしてヨウ化第1銅2.7mg (0.014ミリモル)のジメチルホルムアミド(1.5ml)溶液に、トリメチルシリルアセチレン28.3μl(0.201ミリモル)とトリエチルアミン59.8μl(0.429ミリモル)を加え、50℃で4時間攪拌した。室温まで放冷後水を加え、酢酸エチルを用いて抽出し、飽和食塩水で洗浄後、減圧濃縮した。残渣のメタノール(5ml)溶液に、炭酸カリウム100mg(0.724ミリモル)を加え、室温で1時間攪拌した。水を加え、ジエチルエーテルを用いて抽出し、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物5.5mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 6.89(1H, d), 7.51(1H, d), 7.83(1H, d), 8.21(1H, d)

実施例B205

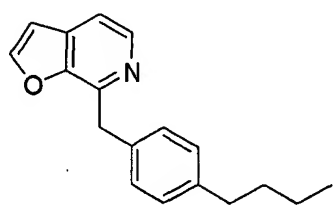
4-ブチルベンジルマグネシウムクロリド



実施例Ｂ１の化合物1.04g (5.69ミリモル)、マグネシウム761mg (31.3ミリモル)そして触媒量の1,2-ジブロモエタンのジエチルエーテル(11ml)の混合液を加熱還流によりイニシエーションした後、熱源を除き、さらに実施例Ｂ１の化合物4.16g (22.8ミリモル)のジエチルエーテル60ml溶液を緩やかな還流を保つ速度で滴下し、30分間加熱還流した。室温まで放冷し表題化合物を0.4Mジエチルエーテル溶液として得、そのまま次の反応に用いた。

実施例Ｂ２０６

7-(4-ブチルベンジル)フロ[2,3-c]ピリジン

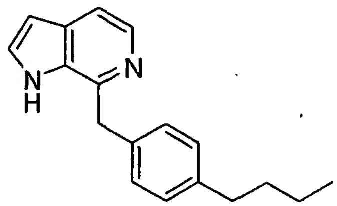


実施例Ｂ２０４の化合物5.0mg (0.033ミリモル)と[1,1'-ビス(ジフェニルホスフィノ)フェロセン]ジクロロニッケル(II)4.5mg (0.0065ミリモル)のテトラヒドロフラン(1ml)溶液に、実施例Ｂ２０５の化合物300μl(0.1ミリモル)を加え、50℃で1時間攪拌した。室温まで放冷後、酢酸エチルを加え、飽和塩化アンモニア水溶液と飽和食塩水で洗浄後、減圧濃縮した。残渣をNH-シリカゲルカラムクロマトグラフィーで精製し、表題化合物2.9mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.89(3H, t), 1.29-1.35(2H, m), 1.50-1.58(2H, m), 2.54(2H, t), 4.40(2H, s), 6.78(1H, d), 7.08(2H, d), 7.30(2H, d), 7.40(1H, d), 7.72(1H, d), 8.34(1H, d)

実施例Ｂ２０７

7-(4-ブチルベンジル)-1H-ピロロ[2,3-c]ピリジン



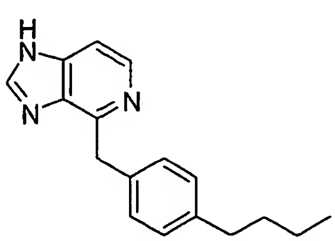
氷冷下、2-クロロ-3-アミノピリジンから特開平7-165708に記載の方法に基づいて合成した1-クロロピロロピリジン19.4mg（0.127ミリモル）とジクロロ(ジフェニルホスフィノプロパン)ニッケル6.9mg(0.013ミリモル)のテトラヒドロフラン(1ml)溶液に、実施例Ｂ２０５の化合物800μl(0.3ミリモル)を加え、加熱還流下4時間攪拌した。室温まで放冷後、酢酸エチルを加え、飽和塩化アンモニア水溶液と飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物7.1mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.91(3H, t), 1.31-1.37(2H, m), 1.55-1.59(2H, m), 2.58(2H, t), 4.44(2H, s), 6.50(1H, d), 7.12(2H, d), 7.18(1H, d), 7.22(2H, d), 7.45(1H, d), 8.21(1H, d)

NHのプロトンは、NMRのチャート上観測されていない。

実施例Ｂ２０８

4-(4-ブチルベンジル)-1-イミダゾ[4,5-c]ピリジン



4-アミノ-2-クロロピリジンからJ.Heterocycl.chem.,2,196(1965)の文献記載の方法に基づいて合成した1-クロロイミダゾピリジン88.6mg（0.577ミリモル）とジクロロ(ジフェニルホスフィノプロパン)ニッケル31.3

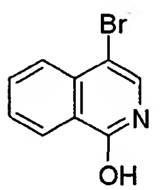
mg (0.0577ミリモル)のテトラヒドロフラン(2ml)溶液に、実施例B205の化合物3.45ml(1.38ミリモル)を加え、加熱還流下2時間攪拌した。室温まで放冷後、酢酸エチルを加え、シリカゲルを用いて濾過し、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物64.2mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.86(3H, t), 1.23-1.32(2H, m), 1.44-1.52(2H, m), 2.47(2H, t), 4.56(2H, s), 7.02(2H, d), 7.19(2H, d), 7.34(1H, d), 8.00(1H, s), 8.25-8.27(1H, m)

NHのプロトンは、NMRのチャート上観測されていない。

実施例B209

4-ブロモ-1-イソキノリノール

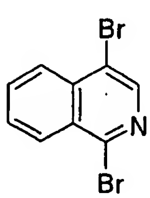


氷冷した1-ヒドロキシイソキノリン5.01g (34.5ミリモル)の酢酸(50ml)溶液に、臭素1.78ml (34.5ミリモル)を加え、室温で2時間攪拌した。その反応溶液に水、酢酸エチルそしてテトラヒドロフランを加え、濾紙を用いて濾過した。有機層を飽和食塩水で洗浄後、減圧濃縮した。残渣を酢酸エチルとヘキサンを用いて再結晶し、表題化合物6.19gを得た。

^{1}H-NMR(DMSO-d6)δ(ppm): 7.56(1H, s), 7.59-7.63(1H, m), 7.76-7.78(1H, m), 7.84-7.89(1H, m), 8.23-8.26(1H, m), 11.59(1H, br s)

実施例B210

1,4-ジブロモイソキノリン

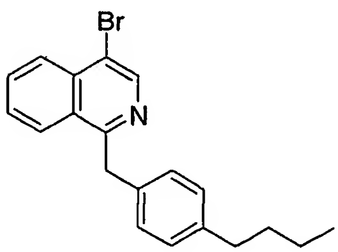


実施例Ｂ２０９の化合物1.40g (8.06ミリモル)と3臭化リン6mlの混合液を150℃で１時間攪拌した後、さらに１時間加熱還流した。室温まで放冷後、その反応溶液を氷に注ぎ、室温まで昇温させた。酢酸エチルを加え、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物845mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 7.76-7.80(1H, m), 7.86-7.90(1H, m), 8.19(1H, d), 8.31-8.34(1H, m), 8.48(1H, s)

実施例Ｂ２１１

4-ブロモ-1-(4-ブチルベンジル)イソキノリン

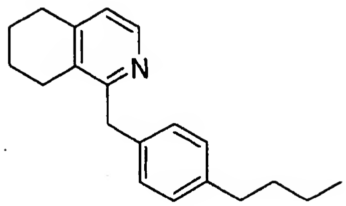


実施例Ｂ２１０の化合物200mg (0.697ミリモル)と[1,1'-ビス(ジフェニルホスフィノ)フェロセン]ジクロロニッケル(II)75.6mg (0.139ミリモル)のテトラヒドロフラン(2ml)溶液に、実施例Ｂ２０５の化合物2.5ml(1ミリモル)を加え、室温で30分間攪拌した。酢酸エチルを加え、飽和塩化アンモニア水溶液、飽和炭酸水素ナトリウム水溶液そして飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物98mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.89(3H, t), 1.29-1.34(2H, m), 1.51-1.60(2H, m), 2.53(2H, t), 4.59(2H, s), 7.06(2H, d), 7.16(2H, d), 7.57-7.61(1H, m), 7.73-7.77(1H, m), 8.15-8.19(2H, m), 8.69(1H, s)

実施例Ｂ２１２

1-(4-ブチルベンジル)-5,6,7,8-テトラヒドロイソキノリン

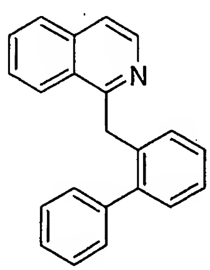


実施例Ｂ２１１の化合物13.0mg (0.0367ミリモル)を酢酸エチルとメタノールの混合液(1:1, 1ml)に溶解し、10%パラジウム－炭素(50%含水) 13mgを加え、室温で常圧水素雰囲気下12時間攪拌した。反応系中を窒素置換した後、触媒をセライトを用いて濾去した。得られた濾液を減圧濃縮し、表題化合物8.8mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.90(3H, t), 1.28-1.38(2H, m), 1.52-1.59(2H, m), 1.74-1.82(4H, m), 2.55(2H, t), 2.66(2H, t), 2.81(2H, t), 4.26(2H, s), 7.07-7.15(5H, m), 8.32(1H, d)

実施例Ｂ２１３

1-[2-(フェニル)ベンジル]イソキノリン

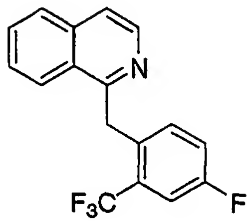


n-ブチルベンジルクロリドの代わりに2-フェニルベンジルブロミドを用いて、実施例Ｂ２と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.62(2H, s), 7.05(1H, d), 7.16(1H, dd), 7.22-7.50(8H, m), 7.52(1H, d), 7.58(1H, dd), 7.65(1H, d), 7.76(1H, d), 8.47(1H, d).

実施例Ｂ２１４

1-[4-フルオロ-2-(トリフルオロメチル)ベンジル] イソキノリン

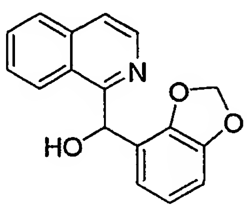


n-ブチルベンジルクロリドの代わりに4-フルオロ-2-(トリフルオロメチル)ベンジルメタンスルホナートを用いて、実施例B2と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.83(2H, s), 6.87(1H, dd), 7.01(1H, ddd), 7.43(1H, dd), 7.54(1H, dd), 7.61(1H, d), 7.67(1H, dd), 7.85(1H, d), 7.96(1H, d), 8.49(1H, d).

実施例B215

1,3-ベンゾジオキソイル-4-イル(1-イソキノリル)メタノール

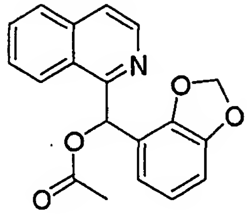


2,3-メチレンジオキシベンズアルデヒドを実施例B82と様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):5.97-5.99(1H, m), 6.09(1H, brs), 6.20-6.40(1H, m), 6.54-6.60(2H, m), 6.65-6.70(2H, m), 7.52(1H, dd), 7.63(1H, d), 7.64(1H, dd), 7.84(1H, d), 8.04(1H, d), 8.53(1H, d).

実施例B216

1,3-ベンゾジオキソイル-4-イル(1-イソキノリル)メチル アセテート

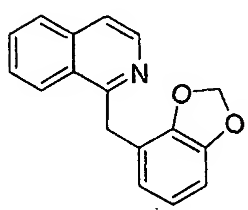


実施例Ｂ２１５の化合物を実施例Ｂ３８と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):2.23(3H, s), 5.98-6.02(2H, m), 6.74-6.79(1H, m), 6.90-6.93(1H, m), 7.15-7.19(1H, m), 7.23-7.28(1H, m), 7.58(1H, dd), 7.60(1H, d), 7.66(1H, dd), 7.83(1H, d), 8.28(1H, d), 8.57(1H, d).

実施例Ｂ２１７

1-(1,3-ベンゾジオキソイル-4-イルメチル)イソキノリン

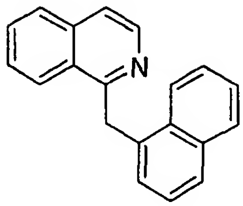


実施例Ｂ２１６の化合物を実施例Ｂ３９と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):4.62(2H, s), 6.02(2H, s), 6.64-6.70(3H, m), 7.57(1H, dd), 7.58(1H, d), 7.66(1H, dd), 7.83(1H, d), 8.23(1H, d), 8.50(1H, d).

実施例Ｂ２１８

1-(1-ナフチルメチル)イソキノリン

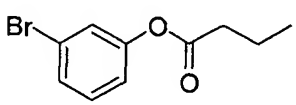


n-ブチルベンジルクロリドの代わりに1-(クロロメチル)ナフタレンを用いて、実施例B2と同様に処理し、表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm):5.13(2H, s), 6.96(1H, d), 7.29(1H, d), 7.45-7.67(5H, m), 7.72(1H, d), 7.84-7.90(2H, m), 8.08(1H, d), 8.26(1H, d), 8.52(1H, d).

実施例B219

3-ブロモフェニルブチレート

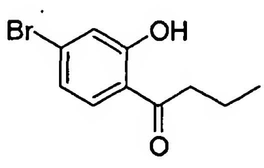


氷冷した3-ブロモフェノール10.0gのピリジン(50ml)溶液に、n-ブチリルクロリド7.25mlを加え、その温度で3時間攪拌した後、室温でさらに3.5時間攪拌した。反応混合物に氷を加え、酢酸エチルで抽出し、1規定塩酸と水で洗浄後、無水硫酸マグネシウムで乾燥後、減圧濃縮した。残査をシリカゲルカラムクロマトグラフィーで精製し表題化合物12.77gを得た。

^{1}H-NMR(CDCl3)δ(ppm): 1.04(3H, t), 1.72-1.82(2H, m), 2.54(2H, t), 7.04(1H, dd), 7.22-7.29(2H, m), 7.36(1H, d).

実施例B220

1-(4-ブロモ-2-ヒドロキシフェニル)-1-ブタノン

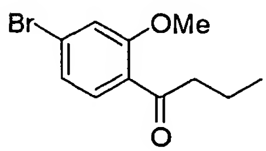


窒素雰囲気下、実施例Ｂ２１９の化合物12.77gのクロロベンゼン(70ml)溶液に塩化アルミニウム10.51gを加え、加熱還流下9時間撹拌した。反応混合物を室温に冷却し、氷を加え酢酸エチルで抽出し、水で洗浄後、無水硫酸マグネシウムで乾燥後、減圧濃縮した。この化合物はさらに精製することなく次反応に用いた。

^{1}H-NMR(CDCl3)δ(ppm): 0.91(3H, t), 1.53-1.65(2H, m), 3.00(2H, t), 7.02(1H, dd), 7.19(1H, d), 7.78(1H, d), 12.50(1H, s).

実施例Ｂ２２１

1-(4-ブロモ-2-メトキシフェニル)-1-ブタノン

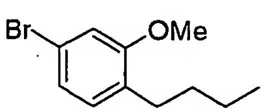


実施例Ｂ２２０の化合物13.30gのアセトン(75ml)溶液に、炭酸カリウム9.07gとヨウ化メチル3.92mlを加え、加熱還流下4時間撹拌した。反応混合物をセライトを用いて濾過し、エーテルを加え不溶物を濾過し、濾液を減圧濃縮した。残査をシリカゲルカラムクロマトグラフィーで精製し、表題化合物9.52gを得た。

^{1}H-NMR(CDCl3)δ(ppm): 0.95(3H, t), 1.64-1.74(2H, m), 2.91(2H, t), 3.90(3H, s), 7.10(1H, d), 7.14(1H, dd), 7.54(1H, d).

実施例Ｂ２２２

4-ブロモ-1-ブチル-2-メトキシベンゼン



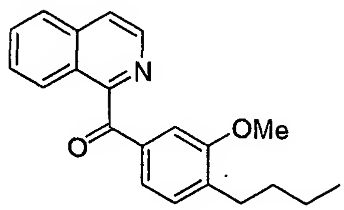
実施例Ｂ２２１の化合物を実施例Ｂ３と同様に還元し、表題化合物を得た。

^{1}H-NMR(CDCl3)δ(ppm): 0.92(3H, t),1.29-1.39(2H, m), 1.48-1.56

(2H, m), 2.54(2H, t), 3.81(3H, s), 6.95(1H, s), 6.96-7.02(2H, m).

実施例B223

(4-ブチル-3-メトキシフェニル)(1-イソキノリル)ケトン

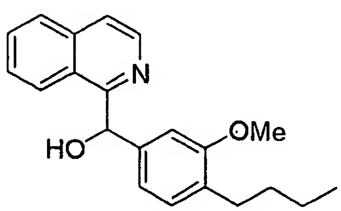


実施例B222の化合物を実施例B36と同様に処理し、表題化合物を含む混合物として得た。

この混合物は分離精製することなく次の反応に用いた。

実施例B224

(4-ブチル-3-メトキシフェニル)(1-イソキノリル)メタノール

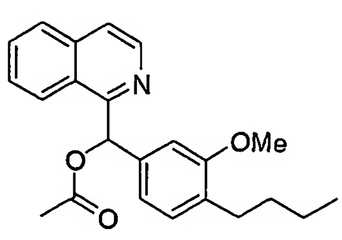


実施例B223の化合物を実施例B37と同様に処理し、表題化合物を含む混合物として得た。

この混合物は分離精製することなく次の反応に用いた。

実施例B225

(4-ブチル-3-メトキシフェニル)(1-イソキノリル)メチル アセテート

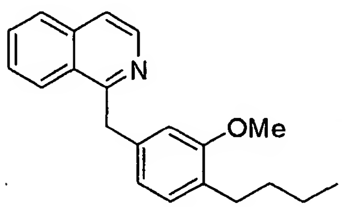


実施例Ｂ２２４の化合物を実施例Ｂ３８と同様に処理し、表題化合物を得た。

^{1}H-NMR(CDCl3)δ(ppm): 0.90(3H, t), 1.24-1.38(2H, m), 1.46-1.60(2H, m), 2.24(3H, s), 2.54(2H, t), 3.76(3H, s), 6.97(1H, s), 6.98(1H, d), 7.06(1H, d), 7.53-7.67(4H, m), 7.83(1H, d), 8.26(1H, d), 8.58(1H, d).

実施例Ｂ２２６

1-(4-ブチル-3-メトキシベンジル)イソキノリン

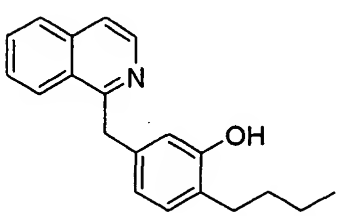


実施例Ｂ２２５の化合物を実施例Ｂ３９と同様に処理し、表題化合物を得た。

^{1}H-NMR(CDCl3)δ(ppm): 0.89(3H, t), 1.27-1.38(2H, t), 1.45-1.54(2H, t), 2.52(2H, t), 3.72(3H, s), 4.63(2H, s), 6.78(1H, d), 6.79(1H, s), 6.99(1H, d), 7.53(1H, dd), 7.55(1H, d), 7.64(1H, dd), 7.80(1H, d), 8.19(1H, d), 8.49(1H, d).

実施例Ｂ２２７

2-ブチル-5-(1-イソキノリルメチル)フェノール



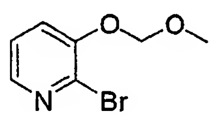
実施例Ｂ２２６の化合物を実施例Ｂ４０と同様に処理し、表題化合物を得た。

^{1}H-NMR(CDCl3)δ(ppm): 0.91(3H, t), 1.30-1.40(2H, m), 1.52-1.65(2H, m), 2.55(2H, t), 4.55(2H, s), 6.46(1H, brs), 6.85(1H, d), 7.03(1H, d), 7.32-7.40(1H, m), 7.55(1H, dd), 7.68(1H, dd), 7.81(1H, d), 7.94-8.05(1H, m), 8.14(1H, d).

フェノール性水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ２２８

2-ブロモ-3-(メトキシメトキシ)ピリジン

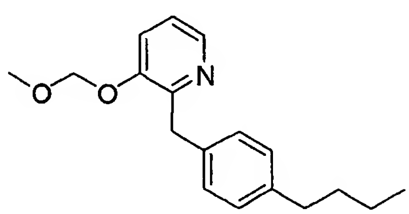


2-ブロモ-3-ヒドロキシピリジンを用い、実施例Ｂ２０２と同様に合成した。

^{1}H-NMR($CDCl_3$)δ(ppm): 3.53(3H, s), 5.29(2H, s), 7.19-7.23(1H, m), 7.42-7.45(1H, m), 8.04-8.06(1H, m)

実施例Ｂ２２９

2-(4-ブチルベンジル)-3-(メトキシメトキシ)ピリジン



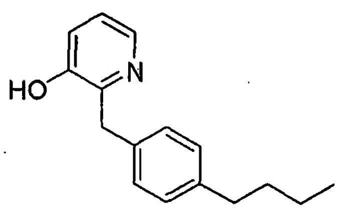
氷冷した実施例Ｂ２２８の化合物524mg (2.40ミリモル)とジクロロ(ジフェニルホスフィノプロパン)ニッケル65.0mg (0.120ミリモル)のテトラヒドロフラン(10ml)混合溶液に、実施例Ｂ２０５の化合物7ml(3ミリモル)を加え、加熱還流下5時間攪拌した。室温まで放冷後、酢酸エチルを加え、飽和塩化アンモニア水溶液、飽和炭酸水素ナトリウム水溶液そして飽和食塩水で洗浄後、減圧濃縮した。残渣をNH-シリカゲルを用

いて濾過した。減圧濃縮した後、残渣をメタノール(15ml)に溶解し、トリエチルアミン500μl(3.59ミリモル)と10%パラジウムー炭素(50%含水)50mgを加え、室温で常圧水素雰囲気下3時間攪拌した。反応系中を窒素置換した後、セライトを用いて触媒を濾去し、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物280mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.89(3H, t), 1.28-1.34(2H, m), 1.52-1.58(2H, m), 2.53(2H, t), 3.33(3H, s), 4.16(2H, s), 5.16(2H, s), 7.04-7.10(3H, m), 7.20(2H, d), 7.33-7.35(1H, m), 8.19-8.20(1H, m)

実施例Ｂ２３０

2-(4-ブチルベンジル)-3-ピリジノール



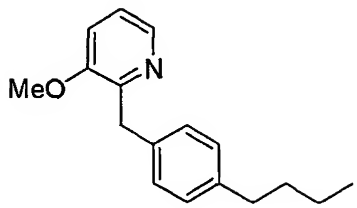
実施例Ｂ２２９の化合物256mg (0.849ミリモル)の塩化メチレン(5ml)溶液に、トリフルオロ酢酸1mlを加え、室温で終夜攪拌した。反応溶液に飽和炭酸水素ナトリウム水溶液、酢酸エチルを加え、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物182mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.58(2H, m), 2.54(2H, t), 4.20(2H, s), 7.02-7.08(4H, m), 7.22(2H, d), 8.08-8.09(1H, m)

フェノール性水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ２３１

2-(4-ブチルベンジル)-3-メトキシピリジン

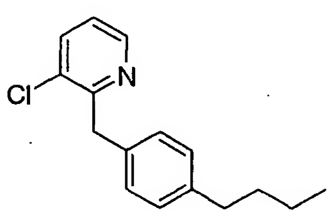


実施例Ｂ２３０の化合物19.2mg (0.0796ミリモル)のアセトン(1ml)溶液に、炭酸カリウム33.0mg (0.239ミリモル)とヨウ化メチル14.9μl (0.239ミリモル)を加え、室温で3時間攪拌した。その反応溶液に酢酸エチルを加え、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物1.47mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.90(3H, t), 1.32-1.34(2H, m), 1.53-1.57(2H, m), 2.54(2H, t), 3.82(3H, s), 4.14(2H, s), 7.06(2H, d), 7.10-7.11(2H, m), 7.21(2H, d), 8.12-8.14(1H, m)

実施例Ｂ２３２

2-(4-ブチルベンジル)-3-クロロピリジン



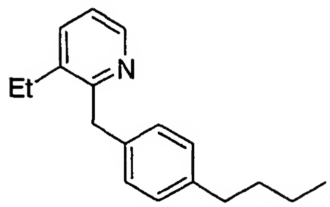
氷冷した2,3-ジクロロピリジン525mg (3.55ミリモル)とジクロロ(ジフェニルホスフィノプロパン)ニッケル96.2mg (0.178ミリモル)のテトラヒドロフラン(4ml)混合液に、実施例Ｂ２０５の化合物12ml(5ミリモル)を加え、室温で1時間攪拌した。反応液に酢酸エチルを加え、飽和塩化アンモニア水溶液、飽和炭酸水素ナトリウム水溶液そして飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物199mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.91(3H, t), 1.29-1.38(2H, m), 1.52-1.60

(2H, m), 2.56(2H, t), 4.28(2H, s), 7.08-7.13(3H, m), 7.21(2H, d), 7.64(1H, dd), 8.46(1H, dd)

実施例Ｂ２３３

2-(4-ブチルベンジル)-3-エチルピリジン

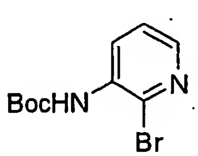


実施例Ｂ２３２の化合物12.9mg（0.0496ミリモル)とジクロロ(ジフェニルホスフィノフェロセン)ニッケル3.4mg（0.0050ミリモル)のテトラヒドロフラン(1ml)混合液に、0.97Mエチルマグネシウムクロリド102μl(0.993ミリモル)を加え、50℃で1時間攪拌し、さらに2時間加熱還流した。室温まで放冷後、その反応溶液に酢酸エチルを加え、飽和塩化アンモニア水溶液と飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物3.29mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.90-0.93(6H, m), 1.30-1.37(2H, m), 1.54-1.59(2H, m), 2.55-2.59(4H, m), 4.12(2H, s), 7.05-7.18(5H, m), 7.55-7.59(1H, m), 8.53-8.55(1H, m)

実施例Ｂ２３４

tert-ブチル *N*-(2-ブロモ-3-ピリジル)カルバメート



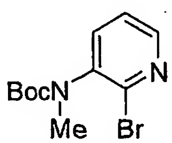
氷冷した3-アミノピリジン3.97g（42.2ミリモル)のジメチルホルムアミド(25ml)混合液に、*N*-ブロモコハク酸イミド7.51g(42.2ミリモル)を加え、その温度で30分間攪拌した。その反応溶液に酢酸エチルを加え、

飽和食塩水で洗浄後、減圧濃縮した。残渣の塩化メチレン(20ml)溶液を氷冷した後、トリエチルアミン3.74ml(26.8ミリモル)、触媒量のジメチルアミノピリジンそしてジ-*t*-ブチル ジカーボネート3.08ml(13.4ミリモル)を加え、室温で終夜撹拌した。減圧濃縮した後、残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物344mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 1.55(9H, s), 7.03(1H, brs), 7.25(1H, dd), 8.03(1H, dd), 8.46(1H, d)

実施例Ｂ２３５

2-ブロモ-3-(*N*-t-ブトキシカルボニル-*N*-メチル)アミノピリジン

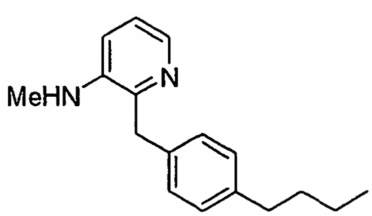


氷冷した実施例Ｂ２３４の化合物344mg (1.26ミリモル)のジメチルホルムアミド(5ml)溶液に、ヨウ化メチル157μl(2.52ミリモル)と66%水素化ナトリウム91.6mg (2.52ミリモル)を加え、その温度で40分間撹拌した。その反応溶液に酢酸エチルを加え、飽和食塩水で洗浄後、シリカゲルを用いて濾過した。有機層を減圧濃縮し、表題化合物356mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 1.36(9H, s), 3.17(3H, s), 7.30(1H, dd), 7.55(1H, d), 8.30(1H, dd)

実施例Ｂ２３６

N-[2-(4-ブチルベンジル)-3-ピリジル]-*N*-メチルアミン



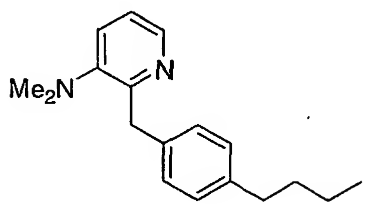
実施例Ｂ２３５の化合物62.8mg (0.219ミリモル)を用い、実施例Ｂ２

11と同様にして4-ブチルベンジル基を導入することにより得られた化合物の塩化メチレン(2ml)溶液に、トリフルオロ酢酸2mlを加え、室温で1時間攪拌した。反応液を炭酸水素ナトリウム水溶液中に滴下し、酢酸エチルを加え、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物29.7mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.91(3H, t), 1.29-1.38(2H, m), 1.53-1.60(2H, m), 2.56(2H, t), 2.72(3H, s), 3.63(1H, br s), 4.09(2H, s), 6.86(1H, d), 7.08-7.12(5H, m), 7.98(1H, dd)

実施例B237

N-[2-(4-ブチルベンジル)-3ピリジル]-*N*,*N*-ジメチルアミン

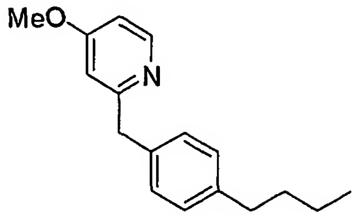


氷冷した実施例B236の化合物26.8mg (0.105ミリモル)の塩化メチレン(2ml)溶液に、酢酸12.1μl(0.211ミリモル)、37%ホルマリン15.8μl(0.211ミリモル)そしてトリアセトキシ水素化ホウ素ナトリウム44.7mg (0.211ミリモル)を加え、室温で30分間攪拌した。酢酸エチルを加え、飽和炭酸水素ナトリウム水溶液と飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物23.3mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.91(3H, t), 1.30-1.36(2H, m), 1.52-1.59(2H, m), 2.55(2H, t), 2.67(6H, s), 4.24(2H, s), 7.06(2H, d), 7.10(1H, dd), 7.18(2H, d), 7.40(1H, dd), 8.27(1H, dd)

実施例B238

2-(4-ブチルベンジル)-4-メトキシピリジン

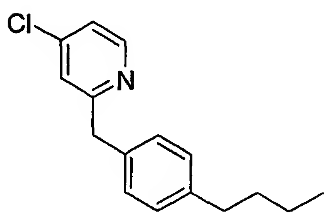


2-クロロ-4-メトキシピリジンを用い、実施例Ｂ２１１と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.91(3H, t), 1.31-1.37(2H, m), 1.53-1.59(2H, m), 2.57(2H, t), 3.78(3H, s), 4.06(2H, s), 6.61-6.65(2H, m), 7.11(2H, d), 7.17(2H, d), 8.36(1H, d)

実施例Ｂ２３９

2-(4-ブチルベンジル)-4-クロロピリジン

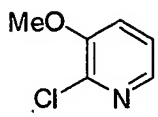


氷冷した実施例Ｂ２３８の化合物52.0mg (0.204ミリモル)のジメチルホルムアミド(1ml)溶液に、オキシ塩化リン57.0μl(0.612ミリモル)を加え、100℃で8時間攪拌した。放冷後、その反応溶液を氷に注ぎ、室温まで昇温した後、酢酸エチルを加え、飽和炭酸水素ナトリウム水溶液と飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物2.29mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.92(3H, t), 1.31-1.38(2H, m), 1.53-1.61(2H, m), 2.59(2H, t), 4.10(2H, s), 7.12-.18(6H, m), 8.44(1H, d)

実施例Ｂ２４０

2-クロロ-3-メトキシピリジン

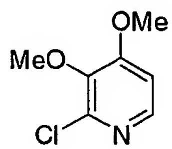


2-クロロ-3-ヒドロキシピリジンを用い、実施例Ｂ２３１と同様にして表題化合物を得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 3.93(3H, s), 7.21-7.22(2H, m), 7.99-8.01(1H, m)

実施例Ｂ２４１

2-クロロ-3,4-ジメトキシピリジン

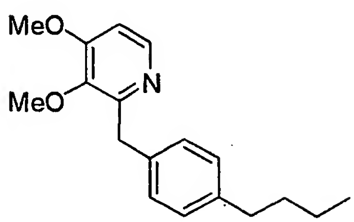


窒素雰囲気下、-78℃に冷却した1.06Mフェニルリチウム シクロペンタン-ジエチルエーテル溶液のテトラヒドロフラン(11ml)溶液に、ジイソプロピルアミン84.0μl(0.599ミリモル)と実施例Ｂ２４０の化合物860mg (5.99ミリモル)のテトラヒドロフラン4ml溶液を加え、-40℃で1時間攪拌した後、さらに-18℃で20分間攪拌した。その反応溶液を-78℃に再冷却した後、トリメトキシボレート2.04ml(18.0ミリモル)を滴下し、0℃で20分間攪拌した。その温度で29%アンモニア水溶液30ml、塩化アンモニウム4.5gそして30%過酸化水素水12mlを順次加え、室温で2時間攪拌した。飽和チオ硫酸ナトリウム、酢酸そして酢酸エチルを加え、飽和食塩水で洗浄した。そしてシリカゲルを用いて濾過して得られた酢酸エチル層を、減圧濃縮した。残渣を用い、実施例Ｂ２３１と同様にして表題化合物31.3mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 3.89(3H, s), 3.94(3H, s), 6.82(1H, d), 8.05(1H, d)

実施例Ｂ２４２

2-(4-ブチルベンジル)-3,4-ジメトキシピリジン

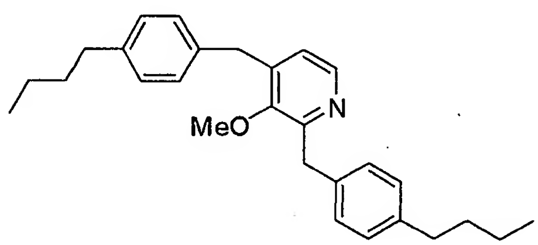


実施例Ｂ２４１の化合物を用い、実施例Ｂ２０６と同様にして表題化合物を得た。

^{1}H-NMR(CDCl$_3$)δ(ppm): 0.90(3H, t), 1.26-1.35(2H, m), 1.53-1.57(2H, m), 2.54(2H, t), 3.70(3H, s), 3.89(3H, s), 4.12(2H, s), 6.72(1H, d), 7.06(2H, d), 7.21(2H, d), 8.20(1H, d)

実施例Ｂ２４３

2,4-ジ(4-ブチルベンジル)-3-メトキシピリジン

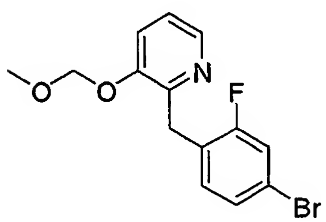


窒素雰囲気下、-78℃に冷却した1.43M t-ブチルリチウムn-ペンタン溶液2.76ml(3.95ミリモル)のジエチルエーテル(5ml)溶液に、実施例Ｂ２４０の化合物436mg (3.04ミリモル)のジエチルエーテル(2ml)溶液を加え、その温度で30分間攪拌した。その反応溶液ににテトラメチルエチレンジアミン688μl(4.56ミリモル)とヘキサクロロエタン719mg(3.04ミリモル)のジエチルエーテル3ml溶液を加え、その温度でさらに1時間攪拌した。徐々に室温まで昇温した後、酢酸エチルを加え、飽和食塩水で洗浄した。そしてシリカゲルを用いて濾過して得られた酢酸エチル層を減圧濃縮した。残渣を用い、実施例Ｂ２０６と同様にして表題化合物10.1mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 0.89-0.94(6H, m), 1.31-1.37(4H, m), 1.52-1.62(4H, m), 2.53-2.59(4H, m), 3.74(3H, s), 4.07(2H, s), 4.13(2H, s), 6.84(1H, d), 6.98(1H, d), 7.04-7.22(8H, m)

実施例Ｂ２４４

2-(4-ブロモ-2-フルオロベンジル)-3-(メトキシメトキシ)ピリジン

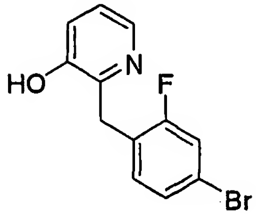


窒素雰囲気下、-78℃に冷却した2.47M n-ブチルリチウムn-ヘキサン溶液862μl(2.13ミリモル)のテトラヒドロフラン(3ml)溶液に、実施例Ｂ２２８の化合物422mg (1.94ミリモル)のテトラヒドロフラン(3ml)溶液を加え、その温度で1時間攪拌した。その反応溶液に臭化第1銅139mg(0.968ミリモル)を加え、0℃で1時間攪拌した後、-78℃に再冷却し、4-ブロモ-2-フルオロベンジルブロミド259mg(0.968ミリモル)を加え、0℃で1時間攪拌した。その溶液にテトラメチルエチレンジアミン584μl(3.88ミリモル)を加え、その温度でさらに1時間攪拌した。反応液にジエチルエーテルとアンモニア水溶液を加え、有機層を飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物81.0mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 3.38(3H, s), 4.17(2H, s), 5.18(2H, s), 7.04(1H, t), 7.11-7.22(3H, m), 7.38(1H, dd), 8.19(1H, dd)

実施例Ｂ２４５

2-(4-ブロモ-2-フルオロベンジル)-3-ピリジノール



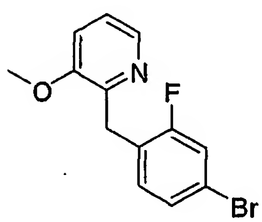
実施例Ｂ２４４の化合物134mg（0.411ミリモル)の塩化メチレン(4ml)にトリフルオロ酢酸1mlを加え、室温で終夜攪拌した。飽和炭酸水素ナトリウム水溶液を用いて中和後、酢酸エチルを加え、酢酸エチル層を飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し表題化合物97.5mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 4.17(2H, s), 7.10-7.24(5H, m), 8.15(1H, t)

フェノール性水酸基のプロトンは、NMRのチャート上観測されていない。

実施例Ｂ２４６

2-(4-ブロモ-2-フルオロベンジル)-3-メトキシピリジン



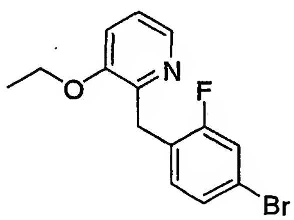
実施例Ｂ２４５の化合物15.8mg（0.0560ミリモル)のジメチルホルムアミド(1ml)溶液に、炭酸カリウム38.7mg（0.280ミリモル)とヨウ化メチル10.5μl（0.168ミリモル)を加え、室温で2時間攪拌した。反応液に酢酸エチルを加え、飽和食塩水で洗浄後、減圧濃縮した。残渣をシリカゲルカラムクロマトグラフィーで精製し、表題化合物14.0mgを得た。

^{1}H-NMR($CDCl_3$)δ(ppm): 3.82(3H, s), 4.15(2H, s), 7.03(1H, t), 7.12-7.22(4H, m), 8.13(1H, dd)

以下の実施例Ｂ化合物は、実施例Ｂ２４６と同様に合成し、精製はLC-MS[溶出溶媒：0.1%トリフルオロ酢酸含有アセトニトリル溶液：0.1%トリフルオロ酢酸含有水溶液＝1:99～100:0/ 20分サイクル、流速：20ml/分、カラム：YMC Combiprep ODS-AM、20mmΦx50mm(Long)]により行った。

実施例Ｂ２４７

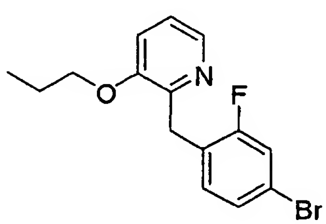
2-(4-ブロモ-2-フルオロベンジル)-3-エトキシピリジン



MS m/z (ESI: MH^+): 310.0

実施例Ｂ２４８

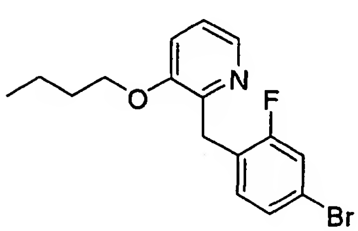
2-(4-ブロモ-2-フルオロベンジル)-3-プロポキシピリジン



MS m/z (ESI: MH^+): 324.0

実施例Ｂ２４９

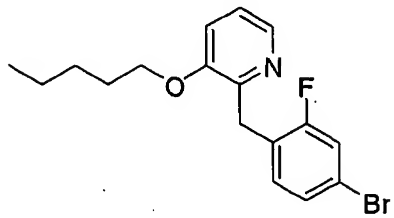
2-(4-ブロモ-2-フルオロベンジル)-3-ブトキシピリジン



MS m/z (ESI: MH^+): 338.1

実施例Ｂ２５０

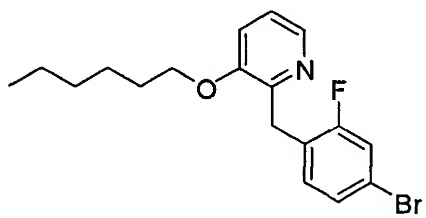
2-(4-ブロモ-2-フルオロベンジル)-3-(ペンチルオキシ)ピリジン



MS m/z (ESI: MH+): 352.1

実施例B251

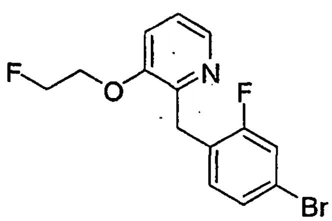
2-(4-ブロモ-2-フルオロベンジル)-3-(ヘキシルオキシ)ピリジン



MS m/z (ESI: MH+): 366.0

実施例B252

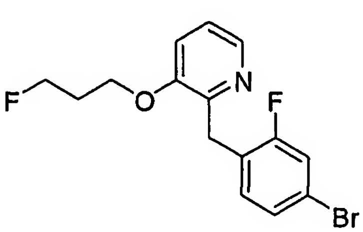
2-(4-ブロモ-2-フルオロベンジル)-3-(2-フルオロエトキシ)ピリジン



MS m/z (ESI: MH+): 328.0

実施例B253

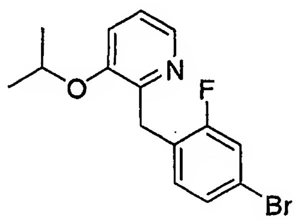
2-(4-ブロモ-2-フルオロベンジル)-3-(3-フルオロプロポキシ)ピリジン



MS *m/z* (ESI: MH^+): 342.0

実施例Ｂ２５４

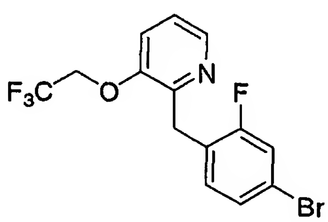
2-(4-ブロモ-2-フルオロベンジル)-3-イソプロポキシピリジン



MS *m/z* (ESI: MH^+): 324.0

実施例Ｂ２５５

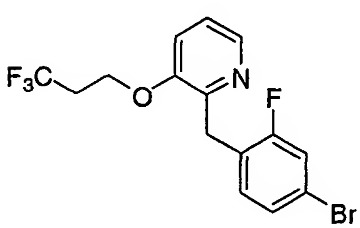
2-(4-ブロモ-2-フルオロベンジル)-3-(2,2,2-トリフルオロエトキシ)ピリジン



MS *m/z* (ESI: MH^+): 364.0

実施例Ｂ２５６

2-(4-ブロモ-2-フルオロベンジル)-3-(3,3,3-トリフルオロプロポキシ)ピリジン



MS *m/z* (ESI: MH^+): 378.0

実施例Ｂ２５７

［実施例Ａ２］に記載したS. cerevisiaeレポーター系を用いて化合

物を評価した。細胞壁画分のセファロスポリナーゼ活性が化合物無処理時の50%以下になる最小濃度をIC50値とした。代表的な化合物の効果を表1に示す。

表1

化合物	IC50 (μg/ml)
1-(4-ブチルベンジル)イソキノリン(実施例B2)	0.39
N1-{3-[4-(1-イソキノリルメチル)フェニル]-2-プロピニル}アセトアミド(実施例B60)	6.25
N1-{3-[4-(1-イソキノリルメチル)フェニル]プロピル}-N1-メチルアセトアミド(実施例B73)	50
5-ブチル-2-(1-イソキノリルメチル)フェノール(実施例B85)	0.20
4-(4-ブチルベンジル)チエノ[3,2-c]ピリジン(実施例B187)	0.78
7-(4-ブチルベンジル)チエノ[2,3-c]ピリジン(実施例B195)	0.39
2-(4-ブチルベンジル)-3-メトキシピリジン(実施例B231)	0.78
2-(4-ブチルベンジル)-3,4-ジメトキシピリジン(実施例B242)	0.78

産業上の利用の可能性

本発明は、GPIアンカー蛋白質の細胞壁への輸送過程に関与する蛋白質をコードする遺伝子を明らかにした。更に本発明は、該蛋白質の活性を阻害する化合物のスクリーニング法も開示し、該阻害活性を持つ代表的な化合物をも開示するものである。

本発明は、GPIアンカー蛋白質の細胞壁への輸送過程を阻害するという、新規メカニズムの抗真菌剤が可能であることを、新規化合物をもって示した。

請求の範囲

１．　真菌における過剰発現により、真菌に対し下記式（Ｉａ）で示される化合物に対する耐性を付与する作用を有する蛋白質をコードする、下記（ａ）から（ｅ）のいずれかに記載のDNA。

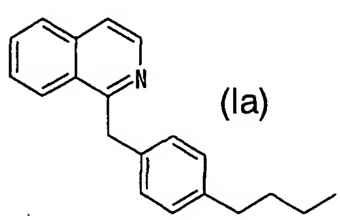
（ａ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列からなる蛋白質をコードするDNA。

（ｂ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列を含むDNA。

（ｃ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列からなるDNAとストリンジェントな条件下でハイブリダイズするDNA。

（ｄ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列において1若しくは複数のアミノ酸が付加、欠失、置換および／または挿入されたアミノ酸配列からなる蛋白質をコードするDNA。

（ｅ）配列番号：２９及び３１あるいは配列番号：２９及び３０をプライマーとして増幅されるDNA。



(Ia)

２．　その機能の欠損により真菌の細胞壁におけるGPIアンカー蛋白質量を減少させる作用を有する蛋白質をコードする、下記（ａ）から（ｅ）のいずれかに記載のDNA。

（ａ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列からなる蛋白質をコードするDNA。

（ｂ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列を含むDNA。

（ｃ）配列番号：１、３、５、２７、３９、４１、５４または５８に記載の塩基配列からなるDNAとストリンジェントな条件下でハイブリダイズするDNA。

（ｄ）配列番号：２、４、６、２８、４０または５９に記載のアミノ酸配列において1若しくは複数のアミノ酸が付加、欠失、置換および／または挿入されたアミノ酸配列からなる蛋白質をコードするDNA。

（ｅ）配列番号：２９及び３１あるいは配列番号：２９及び３０をプライマーとして増幅されるDNA。

３．　請求項１または２に記載のDNAによりコードされる蛋白質。

４．　請求項１または２に記載のDNAが挿入されたベクター。

５．　請求項１または２に記載のDNAまたは請求項４に記載のベクターを保持する形質転換体。

６．　請求項３に記載の蛋白質が過剰発現している真菌である、請求項５に記載の形質転換体。

７．　請求項３に記載の蛋白質の機能が欠損している真菌

８．　請求項５に記載の形質転換体を培養し、該形質転換体またはその培養上清から発現させた蛋白質を回収する工程を含む、請求項３に記載の蛋白質の製造方法。

９．　請求項３に記載の蛋白質に結合する抗体。

１０．　抗真菌作用を有する化合物をスクリーニングする方法であって、

（ａ）請求項３に記載の蛋白質に被検試料を接触させる工程、

（ｂ）該蛋白質と被検試料との結合活性を検出する工程、

（c）該蛋白質に結合する活性を有する化合物を選択する工程、を含む方法。

１１． 抗真菌作用を有する化合物をスクリーニングする方法であって、

（a）請求項３に記載の蛋白質が過剰発現している真菌に被検試料を接触させる工程、

（b)該真菌におけるGPIアンカー蛋白質の細胞壁への輸送量を検出する工程、

（c）請求項３に記載の蛋白質が過剰発現していない真菌に被検試料を接触させた場合と比較して、工程（b）において検出されるGPIアンカー蛋白質の細胞壁への輸送量を減少させる化合物を選択する工程、を含む方法。

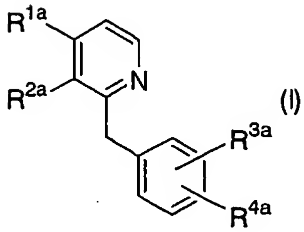
１２． 請求項１０または１１に記載のスクリーニングにより単離しうる、抗真菌作用を有する化合物。

１３． 真菌においてGPIアンカー蛋白質の細胞壁への輸送を阻害する化合物を有効成分とする抗真菌剤。

１４． 請求項９に記載の抗体または請求項１２に記載の化合物を有効成分とする、抗真菌剤。

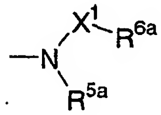
１５．

一般式(I)



[式中R^{1a}およびR^{2a}は同一または相異なってそれぞれ水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、トリフルオロメチル基、トリフルオロメトキシ基、置換されてもよいC_{1-6}アルキル基、C_{2-6}アルケニル基、C

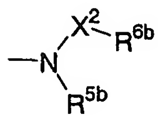
${}_{2-6}$アルキニル基、置換されてもよいC_{1-6}アルコキシ基、または式



（式中X^1は単結合、カルボニル基、または式 $-S(O)_2-$ で表わされる基を意味する；

R^{5a}およびR^{6a}は同一または相異なって、水素原子、または置換されていてもよいC_{1-6}アルキル基を意味する）で表わされる基を示す。また、R^{1a}とR^{2a}は一緒になって、置換されていてもよいベンゼン環、置換されていてもよいピリジン環、置換されていてもよいピロール環、置換されていてもよいチオフェン環、置換されていてもよいフラン環、置換されていてもよいピリダジン環、置換されていてもよいピリミジン環、置換されていてもよいピラジン環、置換されていてもよいイミダゾール環、置換されていてもよいオキサゾール環、置換されていてもよいチアゾール環、置換されていてもよいピラゾール環、置換されていてもよいイソオキサゾール環、置換されていてもよいイソチアゾール環、置換されていてもよいシクロヘキサン環、および置換されていてもよいシクロペンタン環からなる群から選ばれる縮合環の形成してもよい；

R^{3a}、およびR^{4a}は同一または相異なってそれぞれ、水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、カルボキシル基、ホルミル基、ヒドロキシイミノ基、トリフルオロメチル基、トリフルオロメトキシ基、C_{1-6}アルキル基、C_{1-6}アルコキシ基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、式 $-C(O)NR^{7a}R^{7b}$（式中、R^{7a}およびR^{7b}は同一または相異なってそれぞれ水素原子、またはC_{1-6}アルキル基を意味する）、式 $-CO_2R^{7a}$ （式中、R^{7a}は前記定義と同意義を意味する）、式 $-S(O)_nR^{7a}$ （式中、nは０ないし２の整数を意味する。R^{7a}は前記定義と同意義を意味する）、式 $-S(O)_2NR^{7a}R^{7b}$ （式中、R^{7a}およびR^{7b}は前記定義と同意義を意味する）、式



（式中X^2は単結合、カルボニル基、または式 $-S(O)_2-$ で表わされる基を意味する；

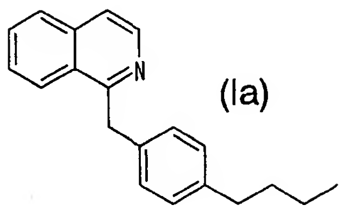
R^{5b}およびR^{6b}は同一または相異なっていて、水素原子、置換されていてもよいC_{1-6}アルキル基、または置換されていてもよいC_{6-14}アリール基を意味する）で表わされる基、または式

$—Z^1—Z^2$

（式中、Z^1は単結合、酸素原子、ビニレン基、またはエチニレン基を意味する；

Z^2は単結合、または０ないし４個の置換基で置換されてもよいC_{1-6}アルキル基を意味する）で表わされる基を意味する。R^{3a}とR^{4a}は一緒になって、メチレンジオキシ基、または1,2-エチレンジオキシ基を意味してもよく、またR^{3a}とR^{4a}は一緒になって、置換されていてもよいベンゼン環、置換されていてもよいピリジン環、置換されていてもよいピロール環、置換されていてもよいチオフェン環、置換されていてもよいフラン環、置換されていてもよいピリダジン環、置換されていてもよいピリミジン環、置換されていてもよいピラジン環、置換されていてもよいイミダゾール環、置換されていてもよいオキサゾール環、置換されていてもよいチアゾール環、置換されていてもよいピラゾール環、置換されていてもよいイソオキサゾール環、置換されていてもよいイソチアゾール環、置換されていてもよいシクロヘキサン環および置換されていてもよいシクロペンタン環からなる群から選ばれる縮合環の形成を意味してもよい。ただし、R^{1a}およびR^{2a}がともに水素原子を意味する場合は除く。〕で示される化合物もしくはその塩またはそれらの水和物を有効成分とする請求項１３に記載の抗真菌剤。

16. 式

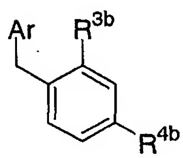


(Ia)

で表される化合物(Ia)を有効成分とする請求項13に記載の抗真菌剤。

17.

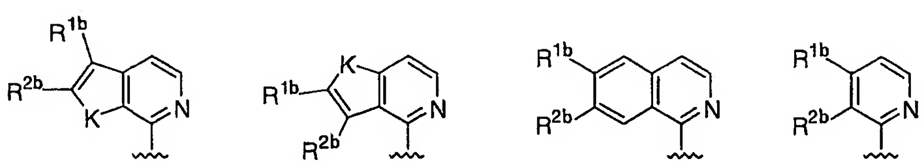
一般式(II)



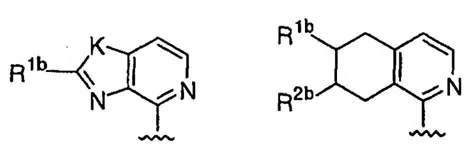
(II)

〔式中Arは下記式(IIIa)－(IIIf)からなる群

(IIIa) (IIIb) (IIIc) (IIId)

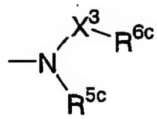


(IIIe) (IIIf)



(式中、Kは硫黄原子、酸素原子、または式 -NH- で表わされる基を意味する；

R^{1b}、R^{2b}は同一または相異なってそれぞれ水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、トリフルオロメチル基、トリフルオロメトキシ基、式



（式中X^3は単結合、カルボニル基、または式 $-S(O)_2-$ で表わされる基を意味する；

R^{5c}およびR^{6c}は同一または相異なっていて、水素原子、置換されていてもよいC_{1-6}アルキル基を意味する）で表わされる基、または式 $-X^4-R^{8a}$（式中、 X^4は、単結合、酸素原子、または硫黄原子を意味する；R^{8a}はC_{1-6}アルキル基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、C_{3-8}シクロアルキル基、またはC_{3-8}シクロアルケニル基を意味する）で表わされる基を示す。また、R^{1b}、R^{2b}は一緒になってメチレンジオキシ基、または1,2-エチレンジオキシ基を形成してもよい。)から選ばれる置換基を意味する；

R^{3b}、およびR^{4b}は同一または相異なってそれぞれ、水素原子、ハロゲン原子、水酸基、ニトロ基、シアノ基、カルボキシル基、ホルミル基、ヒドロキシイミノ基、トリフルオロメチル基、トリフルオロメトキシ基、C_{1-6}アルキル基、C_{1-6}アルコキシ基、C_{2-6}アルケニル基、C_{2-6}アルキニル基、または式、

$—Z^{1b}–Z^{2b}$

（式中、Z^{1b}は単結合、ビニレン基、またはエチニレン基を意味する；

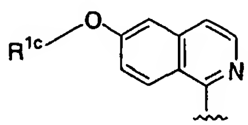
Z^{2b}は単結合、または０ないし４個の置換基で置換されてもよいC_{1-6}アルキル基を意味する）で表わされる基を意味する。；

ただし（１） Arが、R^{1b}およびR^{2b}がともに水素原子である前記式（IIId）で表わされる場合、（２） R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水素原子、メトキシ基、水酸基、メチル基、ベンジルオキシ基、またはハロゲン原子であり、 Arが、R^{1b}およびR^{2b}がともに水素原子またはメトキシ基を意味する前記式（IIIc）で表わされる場合、、（３） R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水

素原子、水酸基、メトキシ基、またはベンジルオキシ基であり、 Arが、R^{1b}およびR^{2b}がともに水酸基またはベンジルオキシ基を意味する前記式(IIIc)で表わされる場合、または（４）Arが、R^{1b}が水素原子でR^{2b}がホルミル基、ヒドロキシメチル基またはメトキシカルボニル基である前記式（IIId）で表わされる場合を除く。〕で示される化合物もしくはその塩またはそれらの水和物

１８．

Arが式、

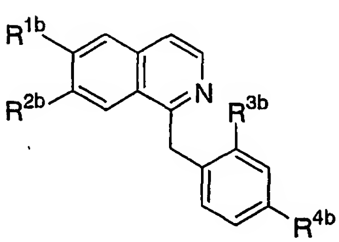


(IIIg)

（式中、R^{1c}が水素原子、置換されてもよいC_{1-6}アルキル基、ベンジル基を意味する）で表わされ、かつ R^{3b}が水素原子を意味する場合を除いた、請求項１７記載の化合物もしくはその塩またはそれらの水和物

１９．

一般式(IIIc2)



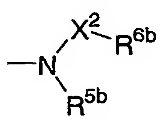
(IIIc2)

〔式中R^{1b}、R^{2b}は前記定義と同意義を意味する。ただし、(１)R^{1b}が式R^{1c}-O-（式中、R^{1c}は前記定義と同意義を意味する）で表わされる基であり、R^{2b}が水素原子であり、R^{3b}が水素原子を意味する場合、（２）R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水素原子、メトキシ基、水酸基、メチル基、ベンジルオキシ基、またはハロゲン原子であり、R^{1b}およびR^{2b}がともに水素原子またはメトキシ基を意味する場合、または

（３） R^{3b}またはR^{4b}の少なくとも一方が水素原子を示し、もう一方が水素原子、水酸基、メトキシ基、またはベンジルオキシ基であり、R^{1b}およびR^{2b}がともに水酸基またはベンジルオキシ基を意味する場合を除く。〕で表される化合物もしくはその塩またはそれらの水和物

２０． 抗真菌作用を有する請求項１７記載の抗真菌剤

２１． R^{3a}、およびR^{4a}のうち少なくとも１つが、式 $-C(O)NR^{7a}R^{7b}$（式中、R^{7a}およびR^{7b}はは前記定義と同意義を意味する）、式 $-CO_2R^{7a}$（式中、R^{7a}は前記定義と同意義を意味する）、式 $-S(O)_nR^{7a}$（式中、nは０ないし２の整数を意味する。R^{7a}は前記定義と同意義を意味する）、式 $-S(O)_2NR^{7a}R^{7b}$（式中、R^{7a}およびR^{7b}は前記定義と同意義を意味する）、式



（式中X^2、R^{5b}およびR^{6b}は前記定義と同意義を意味する）で表わされる基、またはは０ないし４個の置換基で置換されてもよいC_{1-6}アルコキシ基を意味し、またはR^{3a}とR^{4a}は一緒になって、メチレンジオキシ基、または1,2-エチレンジオキシ基を意味する請求項１５記載の抗真菌剤。

２２． 抗真菌作用を有する化合物が、（１）1-ベンジルイソキノリン、（２）1-(4-ブロモベンジル)イソキノリン、（３）1-(4-クロロベンジル)イソキノリン、（４）1-(4-フルオロベンジル)イソキノリン、（５）1-(4-ヨードベンジル)イソキノリン、（６）1-(3-メチルベンジル)イソキノリン、（７）1-(4-メチルベンジル)イソキノリン、（８）1-(3,4-ジメチルベンジル)イソキノリン、（９）1-(3-メトキシベンジル)イソキノリン、（１０）1-(4-メトキシベンジル)イソキノリン、（１１）1-(3,4-メチレンジオキシベンジル)イソキノリン、（１２）1-(4-ベンジルオキシベンジル)イソキノリン、（１３）1-(4-シアノベンジル)イソキノリン、

（１４）1-(4-ニトロベンジル)イソキノリン、（１５）1-(4-アミノベンジル)イソキノリン、（１６）1-(4-メトキシベンジル)-6,7-ジクロロ-イソキノリン、（１７）1-(4-メトキシ-2-ニトロ-ベンジル)-イソキノリン、（１８）1-(4-メトキシベンジル)-6,7-メチレンジオキシ-イソキノリン、（１９）1-(2-アミノ-4-メトキシ-ベンジル)イソキノリン、（２０）1-(4-メトキシベンジル)-7-ヒドロキシ-6-メトキシ-イソキノリン、（２１）1-(4-ベンジルオキシベンジル)-6,7-ジメトキシ-イソキノリン、（２２）1-(4-メトキシベンジル)-6,7-ジメトキシ-イソキノリン、（２３）1-(4-メトキシ-2-ニトロ-ベンジル)-イソキノリン、（２４）3-[4-(1-イソキノリルメチル)フェノキシ]プロピルシアニド、（２５）1-[4-(2,2,3,3-テトラフルオロプロポキシ)ベンジル]イソキノリン、（２６）1-[4-(2-ピペリジノエトキシ)ベンジル]イソキノリン、（２７）4-(1-イソキノリルメチル)フェニル(2-モルフォリノエチル)エーテル、（２８）1-[4-(2-メトキシエトキシ)ベンジル]イソキノリン、（２９）*N*-{2-[4-(1-イソキノリルメチル)フェノキシ]エチル}-*N*,*N*-ジメチルアミン、（３０）1-[4-(フェネチルオキシ)ベンジル]イソキノリン、（３１）1-{4-[(2-メチルアリル)オキシ]ベンジル}イソキノリン、（３２）1-(4-イソブトキシベンジル)イソキノリン、（３３）1-[4-(2-フェノキシエトキシ)ベンジル]イソキノリン、（３４）メチル2-[4-(1-イソキノリルメチル)フェノキシ]アセテート、（３５）2-[4-(1-イソキノリルメチル)フェノキシ]-1-エタノール、（３６）t-ブチル*N*-{2-[4-(1-イソキノリルメチル)フェノキシ]エチル}カーバメート、（３７）1-{4-[3-(テトラヒドロ-2H-2-ピラニルオキシ)プロポキシ]ベンジル}イソキノリン、（３８）2-[4-(1-イソキノリルメチル)フェノキシ] -1-エタンアミン、（３９）1-[4-(3-ピペリジノプロポキシ)ベンジル]イソキノリン、（４９）3-[4-(1-イソキノリルメチル)フェノキシ]-1-プロパノール、（４１）1-[4-(2-

エチルブトキシ)ベンジル]イソキノリン、（４２）4-[4-(1-イソキノリルメチル)フェノキシ]ブタノイックアシッド、（４３）1-(4-{3-[(4-ベンジルピペラジノ)スルフォニル]プロポキシ}ベンジル)イソキノリン、（４４）1-(4-{3-[4-(4-クロロフェニル)ピペラジノ]プロポキシ}ベンジル)イソキノリン、（４５）4-(1-イソキノリルメチル)アニリン、（４６）*N*-[4-(1-イソキノリルメチル)フェニル]ブタンアミド、（４７）*N*-[4-(1-イソキノリルメチル)フェニル]プロパンアミド、（４８）*N*-[4-(1-イソキノリルメチル)フェニル]-1-エタンスルフォンアミド、（４９）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-メチル-エタンスルフォンアミド、（５０）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-メチルアミン、（５１）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-プロピルアミン、または（５２）*N*-[4-(1-イソキノリルメチル)フェニル]-*N*-メチル-*N*-プロピルアミンである請求項１５記載の抗真菌剤。

２３．治効量の請求項１３から２２のいずれかに記載の抗真菌剤を哺乳動物に投与することを含む、真菌感染症の治療方法。

図１

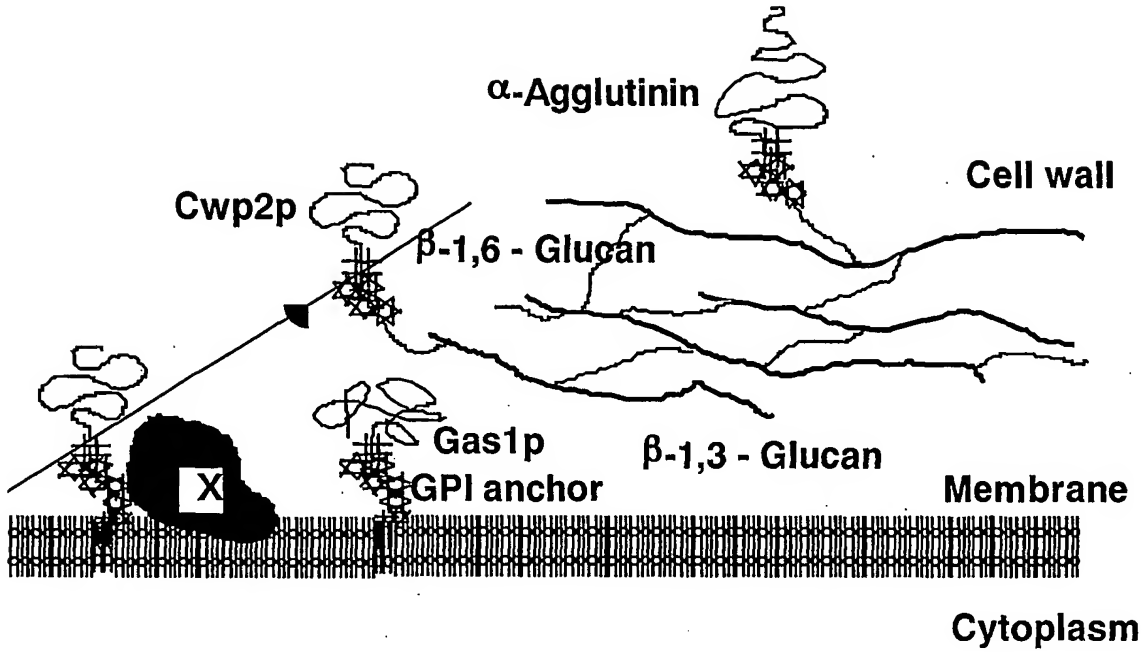

α-Agglutinin
Cell wall
Cwp2p
β-1,6 - Glucan
X
Gas1p
GPI anchor
β-1,3 - Glucan
Membrane
Cytoplasm

図 2

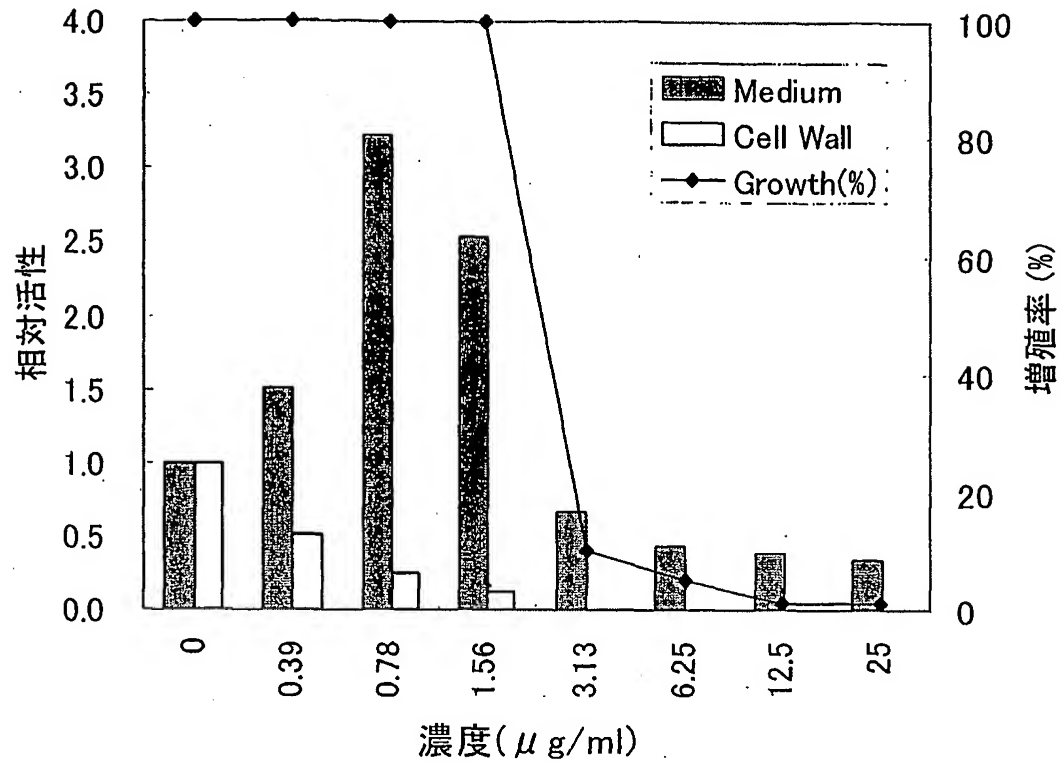
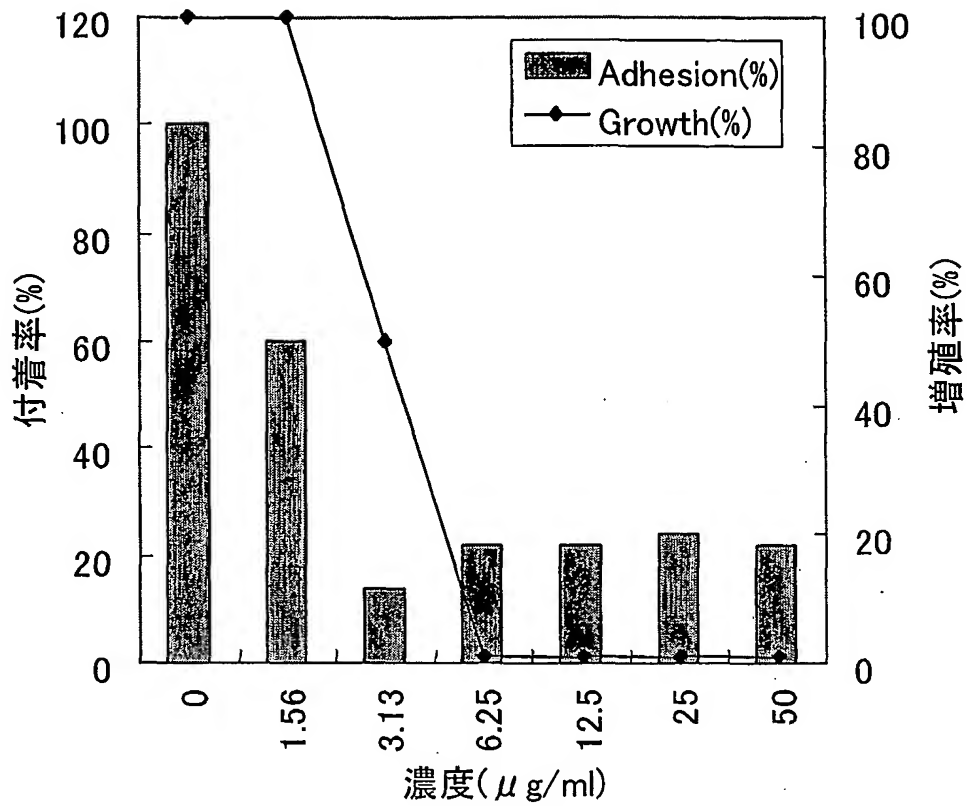

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
相対活性
100
80
60
40
20
0
増殖率 (%)
Medium
Cell Wall
Growth(%)
0
0.39
0.78
1.56
3.13
6.25
12.5
25
濃度(μg/ml)

図 3



120
100
80
60
40
20
0
付着率(%)
100
80
60
40
20
0
増殖率(%)
Adhesion(%)
Growth(%)
0
1.56
3.13
6.25
12.5
25
50
濃度(μg/ml)

図４

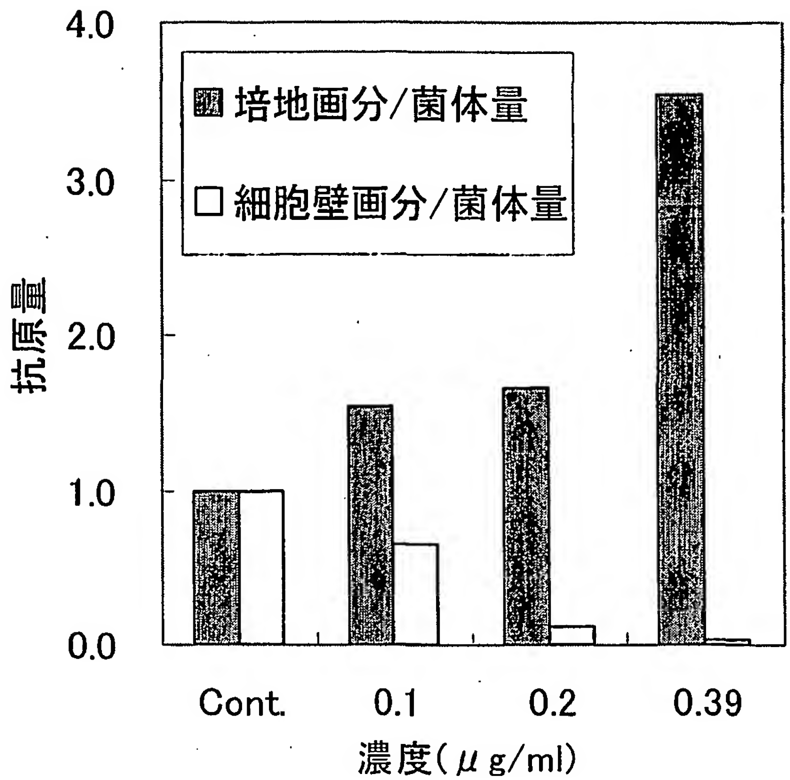


図 5

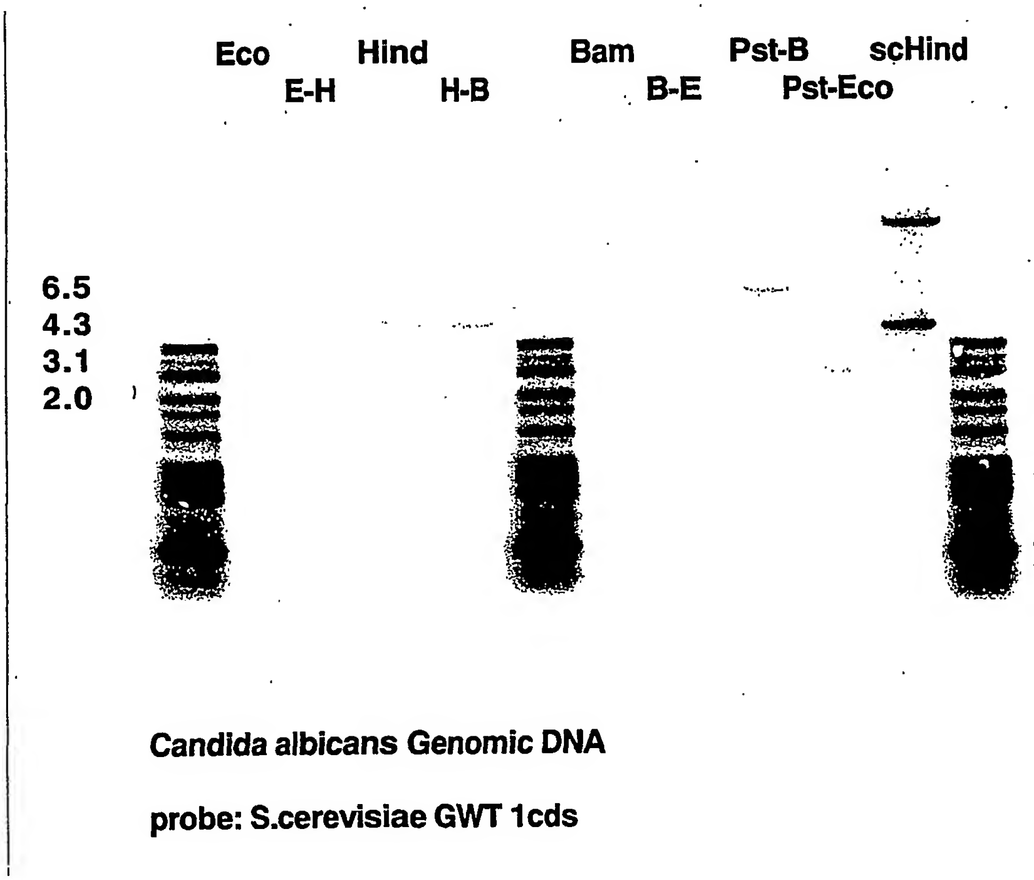
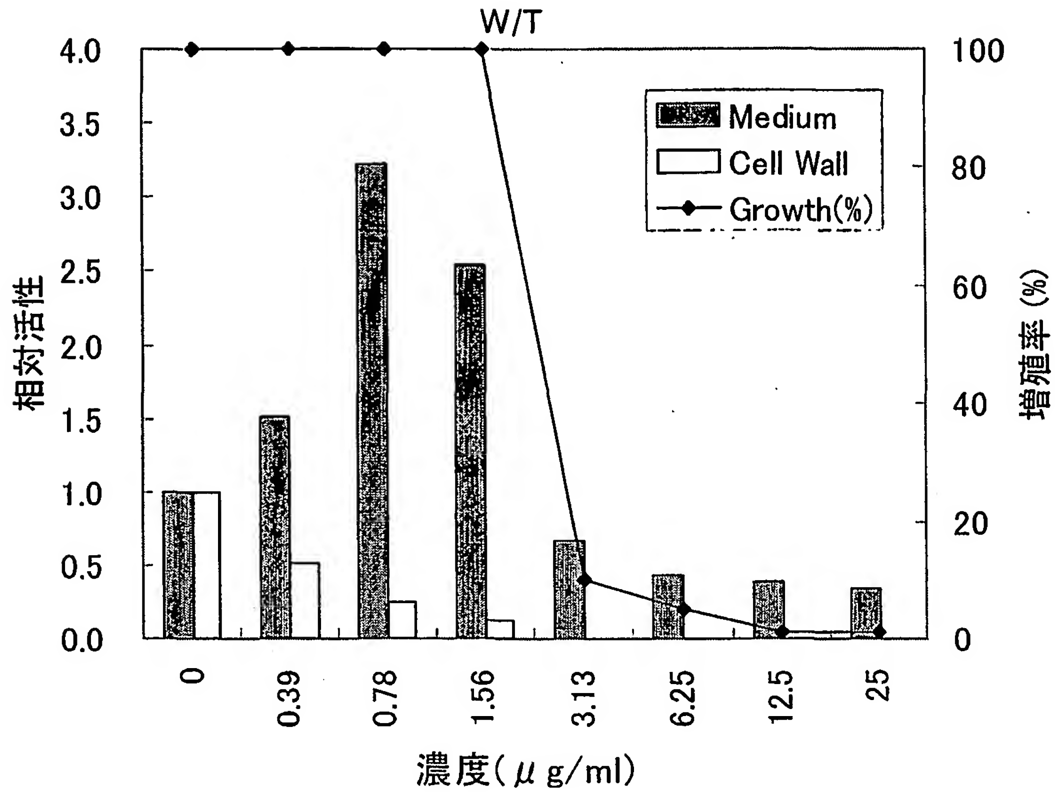

Eco
E-H
Hind
H-B
Bam
B-E
Pst-B
Pst-Eco
scHind
6.5
4.3
3.1
2.0
Candida albicans Genomic DNA
probe: S.cerevisiae GWT 1cds

図 6


W/T
Medium
Cell Wall
Growth(%)
相対活性
増殖率 (%)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
80
60
40
20
0
0
0.39
0.78
1.56
3.13
6.25
12.5
25
濃度(μg/ml)

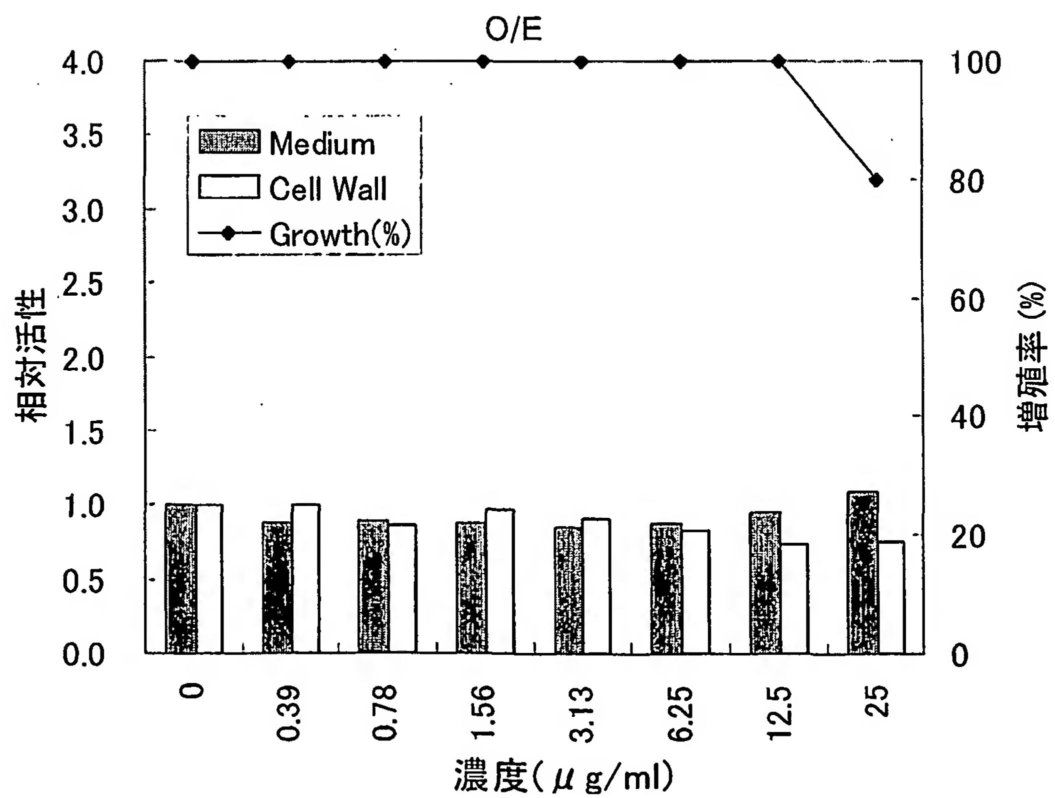

O/E
Medium
Cell Wall
Growth(%)
相対活性
増殖率 (%)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
80
60
40
20
0
0
0.39
0.78
1.56
3.13
6.25
12.5
25
濃度(μg/ml)

図７

〈F-domain〉

S. cerevisiae	ILAVDF PI FP RR F AKVETWG TS L MDLGVGS F
C. albicans	ILAVDF PI FP RR F AKVETWG TS M MDLGVGS F
S. pombe	ILAVDF TL FP RR Y AKVETWG TS L MDLGVGS F

〈R-domain〉

S. cerevisiae	YQEH VT EYG V HWNFF I T
C. albicans	YQEH ET EYG I HWNFF F T
S. pombe	YQEH VS EYG M HWNFF F T

SEQUENCE LISTING

<110> Eisai Co., Ltd.

<120> GPI anchored protein transportor gene GWT1

<130> E1-A0101Y1P

<150> JP 2000-206968

<151> 2000-07-07

<150> JP 2000-316027

<151> 2000-10-17

<160> 63

<170> PatentIn Ver. 2.0

<210> 1

<211> 1497

<212> DNA

<213> Saccharomyces cerevisiae

<220>

<221> CDS

<222> (1)..(1494)

<400> 1

atg gca aca gta cat cag aag aat atg tcg act tta aaa cag aga aaa 48

Met Ala Thr Val His Gln Lys Asn Met Ser Thr Leu Lys Gln Arg Lys

1 5 10 15

gag gac ttt gtg aca ggg ctc aat ggc ggt tct ata aca gaa att aac 96

Glu Asp Phe Val Thr Gly Leu Asn Gly Gly Ser Ile Thr Glu Ile Asn

20 25 30

gca gtg aca tca att gct ttg gta act tac ata tca tgg aac tta ttg 144

Ala Val Thr Ser Ile Ala Leu Val Thr Tyr Ile Ser Trp Asn Leu Leu

35 40 45

aaa aat tcc aac ctt atg cct cct ggc att tcc agc gtg caa tac ata 192

Lys Asn Ser Asn Leu Met Pro Pro Gly Ile Ser Ser Val Gln Tyr Ile

50 55 60

att gat ttt gca ttg aac tgg gtt gct ttg ctt cta tct att act att 240

Ile Asp Phe Ala Leu Asn Trp Val Ala Leu Leu Leu Ser Ile Thr Ile

65 70 75 80

tat gct agt gaa cca tac ctt cta aac acg cta ata ctg tta cct tgt 288

Tyr Ala Ser Glu Pro Tyr Leu Leu Asn Thr Leu Ile Leu Leu Pro Cys

85 90 95

ttg ctc gca ttc ata tat gga aaa ttt act agc tcg agt aaa cct tct 336

Leu Leu Ala Phe Ile Tyr Gly Lys Phe Thr Ser Ser Ser Lys Pro Ser

100 105 110

aat cca ata tac aat aaa aaa aaa atg att aca cag cgg ttc caa cta 384

Asn Pro Ile Tyr Asn Lys Lys Lys Met Ile Thr Gln Arg Phe Gln Leu

115 120 125

gaa aaa aag ccg tat att act gcg tat cgt ggt ggg atg ctt att ctg 432
Glu Lys Lys Pro Tyr Ile Thr Ala Tyr Arg Gly Gly Met Leu Ile Leu
130 135 140

act gct att gcc atc ttg gct gta gat ttt cca att ttc cca agg agg 480
Thr Ala Ile Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro Arg Arg
145 150 155 160

ttt gcc aag gtg gaa act tgg ggg aca tcc ctg atg gat ctt ggt gta 528
Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Leu Met Asp Leu Gly Val
165 170 175

gga tca ttc gtt ttc agt aac ggt att gtt tct tct agg gca ctg ttg 576
Gly Ser Phe Val Phe Ser Asn Gly Ile Val Ser Ser Arg Ala Leu Leu
180 185 190

aaa aac cta agc ttg aag agt aaa ccc agc ttc tta aaa aat gca ttt 624
Lys Asn Leu Ser Leu Lys Ser Lys Pro Ser Phe Leu Lys Asn Ala Phe
195 200 205

aat gcc tta aaa tca gga gga act cta ttg ttc cta gga ttg ctg agg 672
Asn Ala Leu Lys Ser Gly Gly Thr Leu Leu Phe Leu Gly Leu Leu Arg
210 215 220

ttg ttt ttt gta aaa aat ttg gaa tat caa gaa cat gtc aca gaa tat 720
Leu Phe Phe Val Lys Asn Leu Glu Tyr Gln Glu His Val Thr Glu Tyr
225 230 235 240

ggg gtt cat tgg aat ttt ttt atc acc cta tca ttg ttg cca ctt gta 768
Gly Val His Trp Asn Phe Phe Ile Thr Leu Ser Leu Leu Pro Leu Val
245 250 255

ttg acc ttt att gat ccc gtc aca aga atg gtt cca cgc tgc tca att 816
Leu Thr Phe Ile Asp Pro Val Thr Arg Met Val Pro Arg Cys Ser Ile

260 265 270

gca ata ttc att tca tgc att tat gaa tgg cta ctt tta aag gac gat 864

Ala Ile Phe Ile Ser Cys Ile Tyr Glu Trp Leu Leu Leu Lys Asp Asp

275 280 285

cgc act tta aac ttt tta att ttg gct gat aga aat tgt ttc ttc agt 912

Arg Thr Leu Asn Phe Leu Ile Leu Ala Asp Arg Asn Cys Phe Phe Ser

290 295 300

gct aat aga gaa ggc atc ttc tca ttt cta ggt tat tgc tcg att ttt 960

Ala Asn Arg Glu Gly Ile Phe Ser Phe Leu Gly Tyr Cys Ser Ile Phe

305 310 315 320

ctt tgg ggc caa aac acg gga ttt tac ttg ttg gga aat aaa cca act 1008

Leu Trp Gly Gln Asn Thr Gly Phe Tyr Leu Leu Gly Asn Lys Pro Thr

325 330 335

tta aac aat ctt tat aag cct tct acg caa gac gta gtt gca gca tca 1056

Leu Asn Asn Leu Tyr Lys Pro Ser Thr Gln Asp Val Val Ala Ala Ser

340 345 350

aag aag tct tcg act tgg gac tat tgg act tca gta acc cca tta agt 1104

Lys Lys Ser Ser Thr Trp Asp Tyr Trp Thr Ser Val Thr Pro Leu Ser

355 360 365

ggc ctc tgt ata tgg agt aca att ttt ctt gtt atc agc cag ttg gtt 1152

Gly Leu Cys Ile Trp Ser Thr Ile Phe Leu Val Ile Ser Gln Leu Val

370 375 380

ttt caa tac cat cct tat agt gtt tca aga agg ttt gct aac tta cca 1200

Phe Gln Tyr His Pro Tyr Ser Val Ser Arg Arg Phe Ala Asn Leu Pro

385 390 395 400

tat act ttg tgg gtc att act tat aat tta cta ttt ttg act ggg tac 1248

Tyr Thr Leu Trp Val Ile Thr Tyr Asn Leu Leu Phe Leu Thr Gly Tyr
405 410 415

tgc ttg act gac aaa att ttc ggt aat tct tcg gaa tat tat aaa gtt 1296
Cys Leu Thr Asp Lys Ile Phe Gly Asn Ser Ser Glu Tyr Tyr Lys Val
420 425 430

gcc gaa tgc ttg gaa tca atc aac tcc aat ggg ttg ttt tta ttt ttg 1344
Ala Glu Cys Leu Glu Ser Ile Asn Ser Asn Gly Leu Phe Leu Phe Leu
435 440 445

ttg gca aat gtc tct act ggt tta gtc aat atg tct atg gtc acg ata 1392
Leu Ala Asn Val Ser Thr Gly Leu Val Asn Met Ser Met Val Thr Ile
450 455 460

gat tct tca ccc tta aaa tca ttc ctg gtt ttg ttg gca tac tgc tca 1440
Asp Ser Ser Pro Leu Lys Ser Phe Leu Val Leu Leu Ala Tyr Cys Ser
465 470 475 480

ttc ata gct gtc ata tcg gtt ttc ttg tat aga aaa aga ata ttc att 1488
Phe Ile Ala Val Ile Ser Val Phe Leu Tyr Arg Lys Arg Ile Phe Ile
485 490 495

aag cta taa 1497
Lys Leu

<210> 2

<211> 498

<212> PRT

<213> Saccharomyces cerevisiae

<400> 2

Met Ala Thr Val His Gln Lys Asn Met Ser Thr Leu Lys Gln Arg Lys
1 5 10 15
Glu Asp Phe Val Thr Gly Leu Asn Gly Gly Ser Ile Thr Glu Ile Asn
20 25 30
Ala Val Thr Ser Ile Ala Leu Val Thr Tyr Ile Ser Trp Asn Leu Leu
35 40 45
Lys Asn Ser Asn Leu Met Pro Pro Gly Ile Ser Ser Val Gln Tyr Ile
50 55 60
Ile Asp Phe Ala Leu Asn Trp Val Ala Leu Leu Leu Ser Ile Thr Ile
65 70 75 80
Tyr Ala Ser Glu Pro Tyr Leu Leu Asn Thr Leu Ile Leu Leu Pro Cys
85 90 95
Leu Leu Ala Phe Ile Tyr Gly Lys Phe Thr Ser Ser Ser Lys Pro Ser
100 105 110
Asn Pro Ile Tyr Asn Lys Lys Lys Met Ile Thr Gln Arg Phe Gln Leu
115 120 125
Glu Lys Lys Pro Tyr Ile Thr Ala Tyr Arg Gly Gly Met Leu Ile Leu
130 135 140
Thr Ala Ile Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro Arg Arg
145 150 155 160
Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Leu Met Asp Leu Gly Val
165 170 175
Gly Ser Phe Val Phe Ser Asn Gly Ile Val Ser Ser Arg Ala Leu Leu
180 185 190
Lys Asn Leu Ser Leu Lys Ser Lys Pro Ser Phe Leu Lys Asn Ala Phe

195 200 205

Asn Ala Leu Lys Ser Gly Gly Thr Leu Leu Phe Leu Gly Leu Leu Arg

210 215 220

Leu Phe Phe Val Lys Asn Leu Glu Tyr Gln Glu His Val Thr Glu Tyr

225 230 235 240

Gly Val His Trp Asn Phe Phe Ile Thr Leu Ser Leu Leu Pro Leu Val

245 250 255

Leu Thr Phe Ile Asp Pro Val Thr Arg Met Val Pro Arg Cys Ser Ile

260 265 270

Ala Ile Phe Ile Ser Cys Ile Tyr Glu Trp Leu Leu Leu Lys Asp Asp

275 280 285

Arg Thr Leu Asn Phe Leu Ile Leu Ala Asp Arg Asn Cys Phe Phe Ser

290 295 300

Ala Asn Arg Glu Gly Ile Phe Ser Phe Leu Gly Tyr Cys Ser Ile Phe

305 310 315 320

Leu Trp Gly Gln Asn Thr Gly Phe Tyr Leu Leu Gly Asn Lys Pro Thr

325 330 335

Leu Asn Asn Leu Tyr Lys Pro Ser Thr Gln Asp Val Val Ala Ala Ser

340 345 350

Lys Lys Ser Ser Thr Trp Asp Tyr Trp Thr Ser Val Thr Pro Leu Ser

355 360 365

Gly Leu Cys Ile Trp Ser Thr Ile Phe Leu Val Ile Ser Gln Leu Val

370 375 380

Phe Gln Tyr His Pro Tyr Ser Val Ser Arg Arg Phe Ala Asn Leu Pro

385 390 395 400

Tyr Thr Leu Trp Val Ile Thr Tyr Asn Leu Leu Phe Leu Thr Gly Tyr

405 410 415

Cys Leu Thr Asp Lys Ile Phe Gly Asn Ser Ser Glu Tyr Tyr Lys Val

420 425 430

Ala Glu Cys Leu Glu Ser Ile Asn Ser Asn Gly Leu Phe Leu Phe Leu

435 440 445

Leu Ala Asn Val Ser Thr Gly Leu Val Asn Met Ser Met Val Thr Ile

450 455 460

Asp Ser Ser Pro Leu Lys Ser Phe Leu Val Leu Leu Ala Tyr Cys Ser

465 470 475 480

Phe Ile Ala Val Ile Ser Val Phe Leu Tyr Arg Lys Arg Ile Phe Ile

485 490 495

Lys Leu

<210> 3

<211> 1458

<212> DNA

<213> Candida albicans

<220>

<221> CDS

<222> (1)..(1455)

<400> 3

atg tca tcg tct tta aaa caa ttg aaa gaa caa ttt gtc tca gat ttg 48

Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu

1 5 10 15

act ggt ggc aca att gaa gaa att tat gct gta acc agt ata gca tta 96
Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu

20 25 30

tca tct tat ttg tcc ttt aga ttg ttg aaa aag tct ctt ggt gat tta 144
Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu

35 40 45

gct ttg att tac gac tac att ctt aat gtg ttg aca att cta gca tcc 192
Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser

50 55 60

att act gtt tat agc aac agc cct tct tat ttg cat tat ttt att gtt 240
Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val

65 70 75 80

att cca tca tta gtt ata tat cta gtg aat tac cat gtt gag aaa cca 288
Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro

85 90 95

tct tca ccc cat aga caa aat gat aca aaa gaa gat aaa tcg gac gaa 336
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu

100 105 110

cta ttg ccg aga aaa caa ttt ata aca gcc tat cgt tct caa atg ttg 384
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu

115 120 125

ata att act aat cta gct ata tta gct gtt gat ttt cct att ttc cca 432
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro

130 135 140

aga aga ttt gcc aaa gtg gaa aca tgg ggc acg tca atg atg gat tta 480

Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160

gga gtt ggg tcg ttt gtg ttc tcc atg ggg ttg gct aat tct cga caa 528
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175

ttg atc aag aac cac acc gac aac tac aaa ttt agt tgg aag agt tat 576
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190

ttg aaa aca atc aag cag aac ttt atc aag tca gtg cct ata ctt gtt 624
Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205

tta gga gct att cgt ttt gtt agt gtt aag caa ttg gac tat cag gaa 672
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220

cac gaa aca gag tat gga atc cat tgg aat ttt ttc ttc aca tta ggg 720
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240

ttc ttg cca att gta ttg gga ata tta gac ccg gtg ttg aat ttg gtt 768
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255

cca cgc ttc ata ata gga att ggt atc tca att gct tat gag gta gcg 816
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Ala Tyr Glu Val Ala
260 265 270

ttg aat aag act ggt ttg ttg aag ttc att ttg agc agc gaa aac aga 864
Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285

ctt gaa tct ctc atc acc atg aat aaa gaa ggt att ttt tcg ttt att 912
Leu Glu Ser Leu Ile Thr Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300

gga tat ctt tgt att ttt ata att ggt cag tct ttt ggg tca ttt gtt 960
Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305 310 315 320

tta aca ggc tac aaa aca aag aac aac tta ata acc att agc aaa att 1008
Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
325 330 335

cgt att tca aaa aaa caa cac aag aaa gag ctg ctg ctg ttt ttc tca 1056
Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
340 345 350

gtc gcc act act cag gga tta tat ttg gca tgt atc ttc tat cac tta 1104
Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
355 360 365

gct ttc agt ttg ttc atc agc aac tta tca ttc ttg caa cca att tca 1152
Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
370 375 380

aga cga ttg gcc aat ttc ccc tac gtc atg tgg gtc gtt tcg tac aat 1200
Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385 390 395 400

gct acg ttt tta tta tgt tat gac tta att gaa aaa ttt atc ccg ggg 1248
Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
405 410 415

aac ctt act tct act gta ttg gac tct att aat aac aat ggt tta ttt 1296
Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe

420 425 430

atc ttc ttg gtc agc aat tta tta aca ggg ttt att aac atg tcc atc 1344

Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile

435 440 445

aac act ttg gaa act agc aat aaa atg gca gtg att atc ttg att ggc 1392

Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly

450 455 460

tat agt ctt act tgg aca ttg ctc gcc tta tat ttg gat aag agg aag 1440

Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys

465 470 475 480

atc tac atc aag ctt tag 1458

Ile Tyr Ile Lys Leu

485

<210> 4

<211> 485

<212> PRT

<213> Candida albicans

<400> 4

Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu

1 5 10 15

Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu

20 25 30

Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu

35 40 45

Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser

50 55 60

Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val

65 70 75 80

Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro

85 90 95

Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu

100 105 110

Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu

115 120 125

Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro

130 135 140

Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu

145 150 155 160

Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln

165 170 175

Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr

180 185 190

Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val

195 200 205

Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu

210 215 220

His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly

225 230 235 240

Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val

245 250 255

Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Ala Tyr Glu Val Ala

260 265 270

Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg

275 280 285

Leu Glu Ser Leu Ile Thr Met Asn Lys Glu Gly Ile Phe Ser Phe Ile

290 295 300

Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val

305 310 315 320

Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile

325 330 335

Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser

340 345 350

Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu

355 360 365

Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser

370 375 380

Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn

385 390 395 400

Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly

405 410 415

Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe

420 425 430

Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile

435 440 445

Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly

450 455 460

Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys

465 470 475 480

Ile Tyr Ile Lys Leu

485

<210> 5

<211> 1458

<212> DNA

<213> Candida albicans

<220>

<221> CDS

<222> (1)..(1455)

<400> 5

atg tca tcg tct tta aaa caa ttg aaa gaa caa ttt gtc tca gat ttg 48

Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu

1 5 10 15

act ggt ggc aca att gaa gaa att tat gct gta acc agt ata gca tta 96

Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu

20 25 30

tca tct tat ttg tcc ttt aga ttg ttg aaa aag tct ctt ggt gat tta 144

Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu

35 40 45

gct ttg att tac gac tac att ctt aat gtg ttg aca att cta gca tcc 192
Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser
50 55 60
att act gtt tat agc aac agc cct tct tat ttg cat tat ttt att gtt 240
Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val
65 70 75 80
att cca tca tta gtt ata tat cta gtg aat tac cat gtt gag aaa cca 288
Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro
85 90 95
tct tca ccc cat aga caa aat gat aca aaa gaa gat aaa tcg gac gaa 336
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110
cta ttg ccg aga aaa caa ttt ata aca gcc tat cgt tct caa atg ttg 384
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125
ata att act aat cta gct ata tta gct gtt gat ttt cct att ttc cca 432
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140
aga aga ttt gcc aaa gtg gaa aca tgg ggc acg tca atg atg gat tta 480
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160
gga gtt ggg tcg ttt gtg ttc tcc atg ggg ttg gct aat tct cga caa 528
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175
ttg atc aag aac cac acc gac aat tac aaa ttt agt tgg aag agt tat 576
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr

180 185 190

ttg aaa aca atc aag cag aac ttt atc aag tca gtg cct ata ctt gtt 624

Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val

195 200 205

tta gga gct att cgt ttt gtt agt gtt aag caa ttg gac tat cag gaa 672

Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu

210 215 220

cac gaa aca gag tat gga atc cat tgg aat ttt ttc ttc aca tta ggg 720

His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly

225 230 235 240

ttc ttg cca att gta ttg gga ata tta gac ccg gtg ttg aat ttg gtt 768

Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val

245 250 255

cca cgc ttc ata ata gga att ggt atc tca att ggt tat gag gta gcg 816

Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Gly Tyr Glu Val Ala

260 265 270

ttg aat aag act ggt ttg ttg aag ttc att ttg agc agc gaa aac aga 864

Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg

275 280 285

ctt gaa tct ctc atc gcc atg aat aaa gaa ggt att ttt tcg ttt att 912

Leu Glu Ser Leu Ile Ala Met Asn Lys Glu Gly Ile Phe Ser Phe Ile

290 295 300

gga tat ctt tgt att ttt ata att ggt cag tct ttt ggg tca ttt gtt 960

Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val

305 310 315 320

tta aca ggc tac aaa aca aag aac aac tta ata acc att agc aaa att 1008

Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile

325 330 335

cgt att tca aaa aaa caa cac aag aaa gag ctg ctg ctg ttt ttc tca 1056

Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser

340 345 350

gtc gcc act act cag gga tta tat ttg gca tgt atc ttc tat cac tta 1104

Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu

355 360 365

gct ttc agt ttg ttc atc agc aac tta tca ttc ttg caa cca att tca 1152

Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser

370 375 380

aga cga ttg gcc aat ttc ccc tac gtc atg tgg gtc gtt tcg tac aat 1200

Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn

385 390 395 400

gct acg ttt tta tta tgt tat gac tta att gaa aaa ttt atc ccg ggg 1248

Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly

405 410 415

aac ctt act tct act gta ttg gac tct att aat aac aat ggt tta ttt 1296

Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe

420 425 430

atc ttc ttg gtc agc aat tta tta aca ggg ttt att aac atg tcc atc 1344

Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile

435 440 445

aac act ttg gaa act agc aat aaa atg gca gtg att atc ttg att ggc 1392

Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly

450 455 460

tat agt ctt act tgg aca ttg ctc gcc tta tat ttg gat aag agg aag 1440

Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys

465 470 475 480

atc tac atc aag ctt tag 1458

Ile Tyr Ile Lys Leu

485

<210> 6

<211> 485

<212> PRT

<213> Candida albicans

<400> 6

Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu

1 5 10 15

Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu

20 25 30

Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu

35 40 45

Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser

50 55 60

Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val

65 70 75 80

Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro

85 90 95

Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190
Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Gly Tyr Glu Val Ala
260 265 270
Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285
Leu Glu Ser Leu Ile Ala Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300

Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val

305 310 315 320

Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile

325 330 335

Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser

340 345 350

Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu

355 360 365

Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser

370 375 380

Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn

385 390 395 400

Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly

405 410 415

Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe

420 425 430

Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile

435 440 445

Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly

450 455 460

Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys

465 470 475 480

Ile Tyr Ile Lys Leu

485

<210> 7

<211> 1458

<212> DNA

<213> Candida albicans

<400> 7

atgtcatcgt ctttaaaaca attgaaagaa caatttgtct cagatttgac tggtggcaca 60
attgaagaaa tttatgctgt aaccagtata gcattatcat cttatttgtc ctttagattg 120
ttgaaaaagt ctcttggtga tttagctttg atttacgact acattcttaa tgtgttgaca 180
attctagcat ccattactgt ttatagcaac agcccttctt atttgcatta ttttattgtt 240
attccatcat tagttatata tctagtgaat taccatgttg agaaaccatc ttcaccccat 300
agacaaaatg atacaaaaga agataaatcg gacgaactat tgccgagaaa acaatttata 360
acagcctatc gttctcaaat gttgataatt actaatctag ctatattagc tgttgatttt 420
cctattttcc caagaagatt tgccaaagtg gaaacatggg gcacgtcaat gatggattta 480
ggggttgggt cgtttgtgtt ctccatgggg ttggctaatt ctcgacaatt gatcaagaac 540
cacaccgaca actacaaatt tagttggaag agttatttga aaacaatcaa gcagaacttt 600
atcaagtcag tgcctatact tgttttagga gctattcgtt ttgttagtgt taagcaattg 660
gactatcagg aacacgaaac agagtatgga atccattgga atttttcttt cacattaggg 720
ttcttgccaa ttgtattggg aatattagac ccggtgttga atttggttcc acgcttcata 780
ataggaattg gtatctcaat tggttatgag gtagcgttga ataagactgg tttgttgaag 840
ttcattttga gcagcgaaaa cagacttgaa tctctcatcg ccatgaataa agaaggtatt 900
ttttcgttta ttggatatct ttgtattttt ataattggtc agtcttttgg gtcatttgtt 960
ttaacaggct acaaaacaaa gaacaactta ataaccatta gcaaaattcg tatttcaaaa 1020
aaacaacaca agaaagagct gctgctgttt ttctcagtcg ccactactca gggattatat 1080
ttggcatgta tcttctatca cttagctttc agtttgttca tcagcaactt atcattcttg 1140
caaccaattt caagacgatt ggccaatttc ccctacgtca tgtgggtcgt ttcgtacaat 1200

gctacgtttt tattatgtta tgacttaatt gaaaaattta tcccggggaa ccttacttct 1260
actgtattgg attctattaa taacaatggt ttatttatct tcttggtcag caatttatta 1320
acagggttta ttaacatgtc catcaacact ttggaaacta gcaataaaat ggcagtgatt 1380
atcttgattg gctatagtct tacttggaca ttgctcgcct tatatttgga taagaggaag 1440
atctacatca agctttag 1458

<210> 8
<211> 33
<212> DNA
<213> Artificial sequence

<220>
<223> Description of Artificial Sequence:an artificially
synthesized primer sequence

<400> 8
gcagtcgact cgatgaggtc tttgctaatc ttg 33

<210> 9
<211> 33
<212> DNA
<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially
synthesized primer sequence

<400> 9

gcagaattcg acaccacaac cttgaacgta ttg 33

<210> 10

<211> 33

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially
synthesized primer sequence

<400> 10

cccgaattca ctgacggtca aatccaagct act 33

<210> 11

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 11

ggaagctttt ataacaacat agcggcagca gc 32

<210> 12

<211> 49

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 12

cccgcggccg cttgatagta agcttgcttg ggccgcatca tgtaattag 49

<210> 13

<211> 33

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 13

cccggtacca aattaaagcc ttcgagcctc cca 33

<210> 14

<211> 33

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 14

cccggatcct gtttgcagca tgagacttgc ata 33

<210> 15

<211> 45

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 15

cccgcggccg ccccttccaa ttcgaaaacc ttccccagag cagcc 45

<210> 16

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 16

ggttcgaagc cgcaaaaaca gaacaacaaa tt 32

<210> 17

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 17

ggtctagatt gcagtttttc aagaatgcgc ca 32

<210> 18

<211> 33

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 18

gggtctagaa ctgacggtca aatccaagct act 33

<210> 19

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially
synthesized primer sequence

<400> 19
ggaagctttt ataacaacat agcggcagca gc 32

<210> 20
<211> 18
<212> PRT
<213> Candida albicans

<400> 20
Cys Phe Thr Ala Gly Thr Asn Thr Val The Phe Asn Asp Gly Asp Lys
1 5 10 15
Asp Ile
18

<210> 21
<211> 27
<212> DNA
<213> Candida albicans

<400> 21
aaactgttca ctgaacaacc aaatctc 27

<210> 22

<211> 27

<212> DNA

<213> Candida albicans

<400> 22

caactgtacc atttgttaga catcact 27

<210> 23

<211> 30

<212> DNA

<213> Candida albicans

<400> 23

aaacagctgg gatcgcaata agaagacacg 30

<210> 24

<211> 29

<212> DNA

<213> Candida albicans

<400> 24

aaacagctga tggaaatgtg gatggtgtg 29

<210> 25

<211> 60

<212> DNA

<213> Saccharomyces cerevisiae

<400> 25

atggcaacag tacatcagga gaatatgtcg actttaaaac cggatccccg tcgtttaaac 60

<210> 26

<211> 60

<212> DNA

<213> Saccharomyces cerevisiae

<400> 26

ttatagctta atgaatattc tttttctata caagaaaacc gaattcgagc tcgtttaaac 60

<210> 27

<211> 1380

<212> DNA

<213> Schizosaccharomyces pombe

<220>

<221> CDS

<222> (1)..(1380)

<400> 27

atg tca tac aaa ttg gaa aaa gaa gca ttt gtc tca aac ctg acg ggt 48

Met Ser Tyr Lys Leu Glu Lys Glu Ala Phe Val Ser Asn Leu Thr Gly

1 5 10 15

tca agt tcc att gag aca tgt ggc ttg tta tta ata gga att gct tgc 96

Ser Ser Ser Ile Glu Thr Cys Gly Leu Leu Leu Ile Gly Ile Ala Cys

20 25 30

aac gtt ttg tgg gta aac atg act gcg aga aac atc tta ccc aaa ggg 144

Asn Val Leu Trp Val Asn Met Thr Ala Arg Asn Ile Leu Pro Lys Gly

35 40 45

aat ctt ggg ttt ctt gtt gag ttt ttc atc ttt tgc tta att cca tta 192

Asn Leu Gly Phe Leu Val Glu Phe Phe Ile Phe Cys Leu Ile Pro Leu

50 55 60

ttt gtc att tac gtt tca tcg aaa gtt ggc gtt ttc act ctt tgc ata 240

Phe Val Ile Tyr Val Ser Ser Lys Val Gly Val Phe Thr Leu Cys Ile

65 70 75 80

gcc tct ttt ttg cct tcc ttc gtc ctt cat gtt ata agt cca att aat 288

Ala Ser Phe Leu Pro Ser Phe Val Leu His Val Ile Ser Pro Ile Asn

85 90 95

tgg gat gtg ctg aga aga aaa cct ggt tgt tgt ctt act aaa aaa aat 336

Trp Asp Val Leu Arg Arg Lys Pro Gly Cys Cys Leu Thr Lys Lys Asn

100 105 110

gaa aat act ttt gat cga cga att gct gga gtc aca ttt tat cgt tct 384
Glu Asn Thr Phe Asp Arg Arg Ile Ala Gly Val Thr Phe Tyr Arg Ser
115 120 125

caa atg atg ttg gtt act gtc act tgc atc ctg gcc gtt gac ttt acc 432
Gln Met Met Leu Val Thr Val Thr Cys Ile Leu Ala Val Asp Phe Thr
130 135 140

ctt ttc ccg agg aga tat gcc aaa gtt gaa acc tgg gga aca tca ctg 480
Leu Phe Pro Arg Arg Tyr Ala Lys Val Glu Thr Trp Gly Thr Ser Leu
145 150 155 160

atg gat ctt ggt gtt gga tct ttc atg ttt tct tca ggt act gtg gct 528
Met Asp Leu Gly Val Gly Ser Phe Met Phe Ser Ser Gly Thr Val Ala
165 170 175

gga cgg aaa aat gac att aaa aaa cca aat gcg ttt aaa aat gta ttg 576
Gly Arg Lys Asn Asp Ile Lys Lys Pro Asn Ala Phe Lys Asn Val Leu
180 185 190

tgg aat tct ttc atc ctt ttg att tta gga ttt gcg cgc atg ttt tta 624
Trp Asn Ser Phe Ile Leu Leu Ile Leu Gly Phe Ala Arg Met Phe Leu
195 200 205

acg aaa agc atc aat tac caa gaa cat gta agc gaa tat ggc atg cat 672
Thr Lys Ser Ile Asn Tyr Gln Glu His Val Ser Glu Tyr Gly Met His
210 215 220

tgg aac ttt ttt ttc acc cta ggt ttc atg gct ctt ggc gta ttt ttt 720
Trp Asn Phe Phe Phe Thr Leu Gly Phe Met Ala Leu Gly Val Phe Phe
225 230 235 240

ttt cgt cgt tct tta aaa aaa gtc tcc tat ttt aat tta gca acc ttc 768
Phe Arg Arg Ser Leu Lys Lys Val Ser Tyr Phe Asn Leu Ala Thr Phe

245 250 255

att act ctt ctt cat cat tgt ttg ctt gtt tta acc cct ttc caa aaa 816

Ile Thr Leu Leu His His Cys Leu Leu Val Leu Thr Pro Phe Gln Lys

260 265 270

tgg gca cta tcc gcc ccc aga aca aat att ttg gct cag aat aga gag 864

Trp Ala Leu Ser Ala Pro Arg Thr Asn Ile Leu Ala Gln Asn Arg Glu

275 280 285

ggt att gct tct ctt ccc gga tac att gct att tac ttt tat gga atg 912

Gly Ile Ala Ser Leu Pro Gly Tyr Ile Ala Ile Tyr Phe Tyr Gly Met

290 295 300

tat acc ggt agt gta gtt ttg gct gat cga cct cta atg tat act aga 960

Tyr Thr Gly Ser Val Val Leu Ala Asp Arg Pro Leu Met Tyr Thr Arg

305 310 315 320

gct gag tcg tgg aag cgc ttt caa cgt cta tta ttc ccg cta tgc att 1008

Ala Glu Ser Trp Lys Arg Phe Gln Arg Leu Leu Phe Pro Leu Cys Ile

325 330 335

ttg tta gtg ttg tat ctt gtg tct aac ttt ttg tca gtt ggt gtt tct 1056

Leu Leu Val Leu Tyr Leu Val Ser Asn Phe Leu Ser Val Gly Val Ser

340 345 350

cgc cga ctt gct aat acg cct tat gtt gcg aat gtt gcc ttt atc aat 1104

Arg Arg Leu Ala Asn Thr Pro Tyr Val Ala Asn Val Ala Phe Ile Asn

355 360 365

atg ttt ttt ctt act ata tac ata ctt att gat gcc tat tta ttc cca 1152

Met Phe Phe Leu Thr Ile Tyr Ile Leu Ile Asp Ala Tyr Leu Phe Pro

370 375 380

tct tct gtg cca tat gga agt cgc gtc ccc aaa ctg ctt gaa gat gcc 1200

Ser Ser Val Pro Tyr Gly Ser Arg Val Pro Lys Leu Leu Glu Asp Ala

385 390 395 400

aat aat aat ggc ttg ttg gtg ttt ttg att gct aac gtt tta aca gga 1248

Asn Asn Asn Gly Leu Leu Val Phe Leu Ile Ala Asn Val Leu Thr Gly

405 410 415

gta gtt aat tta tcg ttc gac acc ctt cat tct agc aat gca aaa ggc 1296

Val Val Asn Leu Ser Phe Asp Thr Leu His Ser Ser Asn Ala Lys Gly

420 425 430

ttg aca atc atg act atg tat ctt ttt att att tgc tat atg gca cat 1344

Leu Thr Ile Met Thr Met Tyr Leu Phe Ile Ile Cys Tyr Met Ala His

435 440 445

tgg ctt gct caa cac gga att cgt ttt cgc ctt tag 1380

Trp Leu Ala Gln His Gly Ile Arg Phe Arg Leu

450 455 460

<210> 28

<211> 459

<212> PRT

<213> Schizosaccharomyces pombe

<400> 28

Met Ser Tyr Lys Leu Glu Lys Glu Ala Phe Val Ser Asn Leu Thr Gly

1 5 10 15

Ser Ser Ser Ile Glu Thr Cys Gly Leu Leu Leu Ile Gly Ile Ala Cys

20 25 30

Asn Val Leu Trp Val Asn Met Thr Ala Arg Asn Ile Leu Pro Lys Gly
35 40 45
Asn Leu Gly Phe Leu Val Glu Phe Phe Ile Phe Cys Leu Ile Pro Leu
50 55 60
Phe Val Ile Tyr Val Ser Ser Lys Val Gly Val Phe Thr Leu Cys Ile
65 70 75 80
Ala Ser Phe Leu Pro Ser Phe Val Leu His Val Ile Ser Pro Ile Asn
85 90 95
Trp Asp Val Leu Arg Arg Lys Pro Gly Cys Cys Leu Thr Lys Lys Asn
100 105 110
Glu Asn Thr Phe Asp Arg Arg Ile Ala Gly Val Thr Phe Tyr Arg Ser
115 120 125
Gln Met Met Leu Val Thr Val Thr Cys Ile Leu Ala Val Asp Phe Thr
130 135 140
Leu Phe Pro Arg Arg Tyr Ala Lys Val Glu Thr Trp Gly Thr Ser Leu
145 150 155 160
Met Asp Leu Gly Val Gly Ser Phe Met Phe Ser Ser Gly Thr Val Ala
165 170 175
Gly Arg Lys Asn Asp Ile Lys Lys Pro Asn Ala Phe Lys Asn Val Leu
180 185 190
Trp Asn Ser Phe Ile Leu Leu Ile Leu Gly Phe Ala Arg Met Phe Leu
195 200 205
Thr Lys Ser Ile Asn Tyr Gln Glu His Val Ser Glu Tyr Gly Met His
210 215 220
Trp Asn Phe Phe Phe Thr Leu Gly Phe Met Ala Leu Gly Val Phe Phe
225 230 235 240

Phe Arg Arg Ser Leu Lys Lys Val Ser Tyr Phe Asn Leu Ala Thr Phe
245 250 255

Ile Thr Leu Leu His His Cys Leu Leu Val Leu Thr Pro Phe Gln Lys
260 265 270

Trp Ala Leu Ser Ala Pro Arg Thr Asn Ile Leu Ala Gln Asn Arg Glu
275 280 285

Gly Ile Ala Ser Leu Pro Gly Tyr Ile Ala Ile Tyr Phe Tyr Gly Met
290 295 300

Tyr Thr Gly Ser Val Val Leu Ala Asp Arg Pro Leu Met Tyr Thr Arg
305 310 315 320

Ala Glu Ser Trp Lys Arg Phe Gln Arg Leu Leu Phe Pro Leu Cys Ile
325 330 335

Leu Leu Val Leu Tyr Leu Val Ser Asn Phe Leu Ser Val Gly Val Ser
340 345 350

Arg Arg Leu Ala Asn Thr Pro Tyr Val Ala Asn Val Ala Phe Ile Asn
355 360 365

Met Phe Phe Leu Thr Ile Tyr Ile Leu Ile Asp Ala Tyr Leu Phe Pro
370 375 380

Ser Ser Val Pro Tyr Gly Ser Arg Val Pro Lys Leu Leu Glu Asp Ala
385 390 395 400

Asn Asn Asn Gly Leu Leu Val Phe Leu Ile Ala Asn Val Leu Thr Gly
405 410 415

Val Val Asn Leu Ser Phe Asp Thr Leu His Ser Ser Asn Ala Lys Gly
420 425 430

Leu Thr Ile Met Thr Met Tyr Leu Phe Ile Ile Cys Tyr Met Ala His
435 440 445

Trp Leu Ala Gln His Gly Ile Arg Phe Arg Leu

450 455

<210> 29

<211> 35

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<220>

<221> misc_feature

<222> (3)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (9)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (15)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (21)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (24)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (27)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (30)

<223> n represents a, g, c or t.

<400> 29

gcnaargtng aracntgggg nacnwsnytn atgga 35

<210> 30

<211> 38

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially
synthesized primer sequence

<220>

<221> misc_feature

<222> (9)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (12)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (21)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (24)

<223> n represents a, g, c or t.

<400> 30

ttccartgna yncertaytc ngtnacrtgy tcytgrta 38

<210> 31

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<220>

<221> misc_feature

<222> (21)

<223> n represents a, g, c or t.

<220>

<221> misc_feature

<222> (24)

<223> n represents a, g, c or t.

<400> 31

gtraaraara arttccartg nayncertay tc 32

<210> 32

<211> 188

<212> DNA

<213> Aspergillus fumigatus

<400> 32

atggatctgg gcgttggatc gtttgtcttt tcgggcggag tagtatccgc tcgctcacta 60

ctcaagagca ggaccaatgg ctctaaaagg ttgcctcttg ccaagaggtt gattgcgtcg 120

acgcgacact ctattcctct gctcgtcctc ggcctgattc ggctatacag cgtcaaaggc 180

ttggacta 188

<210> 33

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 33

ggagtagtat ccgctcgctc acta 24

<210> 34

<211> 25

<212> DNA

<213> Artificial

<400> 34

gtccaagcct ttgacgctgt atagc 25

<210> 35

<211> 25

<212> DNA

<213> Artificial

<400> 35

gggatgtgct gcaaggcgat taagt 25

<210> 36

<211> 26

<212> DNA

<213> Artificial

<400> 36

tttatgcttc cggctcgtat gttgtg 26

<210> 37

<211> 25

<212> DNA

<213> Artificial

<400> 37

aaaggtgcaa atcccgcggc attga 25

<210> 38

<211> 28

<212> DNA

<213> Artificial

<400> 38

agttcactat atatcttcaa cacaccac 28

<210> 39

<211> 1576

<212> DNA

<213> Aspergillus fumigatus

<220>

<221> CDS

<222> (31)..(1536)

<400> 39

aaggtgcaaa tcccgcggca ttgagtcaag atg gat cca gat tat aaa gct cgc 54

Met Asp Pro Asp Tyr Lys Ala Arg

1 5

aaa gag gcc ttt gtc tca ggt ctt gca gga gga agc atc ctg gaa atc 102

Lys Glu Ala Phe Val Ser Gly Leu Ala Gly Gly Ser Ile Leu Glu Ile

10 15 20

aac gcc gtc acc ttg gtt gct tcg gta tcc gtt ttt ctg tgg tca att 150

Asn Ala Val Thr Leu Val Ala Ser Val Ser Val Phe Leu Trp Ser Ile

25 30 35 40

cta caa tct cgc cta tcc ttt ttc aca ccc tac agc gcc gct gcc ctt 198

Leu Gln Ser Arg Leu Ser Phe Phe Thr Pro Tyr Ser Ala Ala Ala Leu

45 50 55

ctc gtt gat ttc ctg ctc aat gta cta gct atc ttg ttc gca acc act 246

Leu Val Asp Phe Leu Leu Asn Val Leu Ala Ile Leu Phe Ala Thr Thr

60 65 70

tta tac tct tcg gcg cct ctt ctt ctc aat ctc ctt cta ata tct ccc 294

Leu Tyr Ser Ser Ala Pro Leu Leu Leu Asn Leu Leu Leu Ile Ser Pro

75 80 85

gct ctg ctg ata ctc ctc tct acg aaa cgt cct cgg acc ccc gtc aaa 342

Ala Leu Leu Ile Leu Leu Ser Thr Lys Arg Pro Arg Thr Pro Val Lys

90 95 100

gcg aaa cct cct cgc cag tcc gct aga gct ggg aaa gat gac tcg aaa 390
Ala Lys Pro Pro Arg Gln Ser Ala Arg Ala Gly Lys Asp Asp Ser Lys
105 110 115 120
cat gcg aca gcc ttg cca gag tct cta ccc att cat cca ttt ctc acg 438
His Ala Thr Ala Leu Pro Glu Ser Leu Pro Ile His Pro Phe Leu Thr
125 130 135
aca tat cgc gcc gcc atg atg gtt atc acg tgc atc gct atc ttg gct 486
Thr Tyr Arg Ala Ala Met Met Val Ile Thr Cys Ile Ala Ile Leu Ala
140 145 150
gtg gat ttt cgc att ttt cct cgc cga ttc gcc aag gta gaa aac tgg 534
Val Asp Phe Arg Ile Phe Pro Arg Arg Phe Ala Lys Val Glu Asn Trp
155 160 165
ggt aca tca ctc atg gat ctg ggc gtt gga tcg ttt gtc ttt tcg ggc 582
Gly Thr Ser Leu Met Asp Leu Gly Val Gly Ser Phe Val Phe Ser Gly
170 175 180
gga gta gta tcc gct cgc tca cta ctc aag agc agg acc aat ggc tct 630
Gly Val Val Ser Ala Arg Ser Leu Leu Lys Ser Arg Thr Asn Gly Ser
185 190 195 200
aaa agg ttg cct ctt gcc aag agg ttg att gcg tcg acg cga cac tct 678
Lys Arg Leu Pro Leu Ala Lys Arg Leu Ile Ala Ser Thr Arg His Ser
205 210 215
att cct ctg ctc gtc ctc ggc ctg att cgg cta tac agc gtc aaa ggc 726
Ile Pro Leu Leu Val Leu Gly Leu Ile Arg Leu Tyr Ser Val Lys Gly
220 225 230
ttg gac tat gcg gag cac gtc acc gag tac ggc gta cat tgg aac ttc 774
Leu Asp Tyr Ala Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe

235 240 245

ttc ttt aca ttg ggt ctt ttg cct ccg ttc gtg gag gtc ttc gac gcc 822

Phe Phe Thr Leu Gly Leu Leu Pro Pro Phe Val Glu Val Phe Asp Ala

250 255 260

ttg gct acg atc att ccg tca tac gag gtt ctc tcc gtg ggg atc gcc 870

Leu Ala Thr Ile Ile Pro Ser Tyr Glu Val Leu Ser Val Gly Ile Ala

265 270 275 280

gtc ttg tat caa gtt gcc cta gag tca aca gac ttg aaa agc tac atc 918

Val Leu Tyr Gln Val Ala Leu Glu Ser Thr Asp Leu Lys Ser Tyr Ile

285 290 295

ctc gtc tcc cct cgt ggg cca agc tta ctg tcc aag aat cgt gaa ggc 966

Leu Val Ser Pro Arg Gly Pro Ser Leu Leu Ser Lys Asn Arg Glu Gly

300 305 310

gtc ttc tcc ttc tca ggt tat ctc gcg att ttt ctt gct ggt cgt gcg 1014

Val Phe Ser Phe Ser Gly Tyr Leu Ala Ile Phe Leu Ala Gly Arg Ala

315 320 325

atc ggc att cgg ata atc cct cgc gga act tct ttc tca aga agc cca 1062

Ile Gly Ile Arg Ile Ile Pro Arg Gly Thr Ser Phe Ser Arg Ser Pro

330 335 340

gaa cag gcc agg aga cgg gtc ctg atc agc ctt ggc gtg caa gcg tta 1110

Glu Gln Ala Arg Arg Arg Val Leu Ile Ser Leu Gly Val Gln Ala Leu

345 350 355 360

gtg tgg acc act ctt ttt gtg ttg aac tcc act tat gcg atg gga tac 1158

Val Trp Thr Thr Leu Phe Val Leu Asn Ser Thr Tyr Ala Met Gly Tyr

365 370 375

gga gct aat atc cct gtc tcc cgc cgc ctc gct aac atg ccc tat gtc 1206

Gly Ala Asn Ile Pro Val Ser Arg Arg Leu Ala Asn Met Pro Tyr Val
380 385 390

ctt tgg gtt tcg gcg ttc aac acc gcg caa ctg ttt gtg ttc tgc ctg 1254
Leu Trp Val Ser Ala Phe Asn Thr Ala Gln Leu Phe Val Phe Cys Leu
395 400 405

atc gaa aca ctc tgc ttt cct gca gtt cat cgg aca acg act caa gag 1302
Ile Glu Thr Leu Cys Phe Pro Ala Val His Arg Thr Thr Thr Gln Glu
410 415 420

agc gaa tct gag cga gtc gat ttt gct acg agc cga atc atg tcg gcc 1350
Ser Glu Ser Glu Arg Val Asp Phe Ala Thr Ser Arg Ile Met Ser Ala
425 430 435 440

ttc aat aag aac agt ctc gcg atc ttt ctt ttg gcc aat ctt ctg act 1398
Phe Asn Lys Asn Ser Leu Ala Ile Phe Leu Leu Ala Asn Leu Leu Thr
445 450 455

gga gct gtg aat ctg agc atc tcc aca att gat gct aat aca gcg cag 1446
Gly Ala Val Asn Leu Ser Ile Ser Thr Ile Asp Ala Asn Thr Ala Gln
460 465 470

gcc atc gct gtt ctc att gga tat tca tcc att atc aca ggg gtt gct 1494
Ala Ile Ala Val Leu Ile Gly Tyr Ser Ser Ile Ile Thr Gly Val Ala
475 480 485

cta gca ttg cat cat gcc aat atc aaa gta ctt cct ttc tag 1536
Leu Ala Leu His His Ala Asn Ile Lys Val Leu Pro Phe
490 495 500

ggtatttacg agcaattggt ggtgtgttga agatatatag 1576

<210> 40

<211> 501

<212> PRT

<213> Aspergillus fumigatus

<400> 40

Met Asp Pro Asp Tyr Lys Ala Arg Lys Glu Ala Phe Val Ser Gly Leu
1 5 10 15

Ala Gly Gly Ser Ile Leu Glu Ile Asn Ala Val Thr Leu Val Ala Ser
20 25 30

Val Ser Val Phe Leu Trp Ser Ile Leu Gln Ser Arg Leu Ser Phe Phe
35 40 45

Thr Pro Tyr Ser Ala Ala Ala Leu Leu Val Asp Phe Leu Leu Asn Val
50 55 60

Leu Ala Ile Leu Phe Ala Thr Thr Leu Tyr Ser Ser Ala Pro Leu Leu
65 70 75 80

Leu Asn Leu Leu Leu Ile Ser Pro Ala Leu Leu Ile Leu Leu Ser Thr
85 90 95

Lys Arg Pro Arg Thr Pro Val Lys Ala Lys Pro Pro Arg Gln Ser Ala
100 105 110

Arg Ala Gly Lys Asp Asp Ser Lys His Ala Thr Ala Leu Pro Glu Ser
115 120 125

Leu Pro Ile His Pro Phe Leu Thr Thr Tyr Arg Ala Ala Met Met Val
130 135 140

Ile Thr Cys Ile Ala Ile Leu Ala Val Asp Phe Arg Ile Phe Pro Arg
145 150 155 160

Arg Phe Ala Lys Val Glu Asn Trp Gly Thr Ser Leu Met Asp Leu Gly

165 170 175

Val Gly Ser Phe Val Phe Ser Gly Gly Val Val Ser Ala Arg Ser Leu

180 185 190

Leu Lys Ser Arg Thr Asn Gly Ser Lys Arg Leu Pro Leu Ala Lys Arg

195 200 205

Leu Ile Ala Ser Thr Arg His Ser Ile Pro Leu Leu Val Leu Gly Leu

210 215 220

Ile Arg Leu Tyr Ser Val Lys Gly Leu Asp Tyr Ala Glu His Val Thr

225 230 235 240

Glu Tyr Gly Val His Trp Asn Phe Phe Phe Thr Leu Gly Leu Leu Pro

245 250 255

Pro Phe Val Glu Val Phe Asp Ala Leu Ala Thr Ile Ile Pro Ser Tyr

260 265 270

Glu Val Leu Ser Val Gly Ile Ala Val Leu Tyr Gln Val Ala Leu Glu

275 280 285

Ser Thr Asp Leu Lys Ser Tyr Ile Leu Val Ser Pro Arg Gly Pro Ser

290 295 300

Leu Leu Ser Lys Asn Arg Glu Gly Val Phe Ser Phe Ser Gly Tyr Leu

305 310 315 320

Ala Ile Phe Leu Ala Gly Arg Ala Ile Gly Ile Arg Ile Ile Pro Arg

325 330 335

Gly Thr Ser Phe Ser Arg Ser Pro Glu Gln Ala Arg Arg Arg Val Leu

340 345 350

Ile Ser Leu Gly Val Gln Ala Leu Val Trp Thr Thr Leu Phe Val Leu

355 360 365

Asn Ser Thr Tyr Ala Met Gly Tyr Gly Ala Asn Ile Pro Val Ser Arg

370 375 380

Arg Leu Ala Asn Met Pro Tyr Val Leu Trp Val Ser Ala Phe Asn Thr

385 390 395 400

Ala Gln Leu Phe Val Phe Cys Leu Ile Glu Thr Leu Cys Phe Pro Ala

405 410 415

Val His Arg Thr Thr Thr Gln Glu Ser Glu Ser Glu Arg Val Asp Phe

420 425 430

Ala Thr Ser Arg Ile Met Ser Ala Phe Asn Lys Asn Ser Leu Ala Ile

435 440 445

Phe Leu Leu Ala Asn Leu Leu Thr Gly Ala Val Asn Leu Ser Ile Ser

450 455 460

Thr Ile Asp Ala Asn Thr Ala Gln Ala Ile Ala Val Leu Ile Gly Tyr

465 470 475 480

Ser Ser Ile Ile Thr Gly Val Ala Leu Ala Leu His His Ala Asn Ile

485 490 495

Lys Val Leu Pro Phe

500

<210> 41

<211> 1648

<212> DNA

<213> Aspergillus fumigatus

<220>

<221> intron

<222> (122)..(198)

<220>

<221> CDS

<222> (26)..(121)

<220>

<221> CDS

<222> (199)..(1608)

<400> 41

```
gcaaatcccg cggcattgag tcaag atg gat cca gat tat aaa gct cgc aaa    52
                            Met Asp Pro Asp Tyr Lys Ala Arg Lys
                             1               5

gag gcc ttt gtc tca ggt ctt gca gga gga agc atc ctg gaa atc aac   100
Glu Ala Phe Val Ser Gly Leu Ala Gly Gly Ser Ile Leu Glu Ile Asn
 10                  15                  20                  25

gcc gtc acc ttg gtt gct tcg gttcgtgtta ctatcttatt gtggctactt      151
Ala Val Thr Leu Val Ala Ser
                 30

cgcctacatt gtttctcgac taaccgagtc tctttgcgat caatcag gta tcc gtt   207
                                                    Val Ser Val
```

35

ttt ctg tgg tca att cta caa tct cgc cta tcc ttt ttc aca ccc tac 255
Phe Leu Trp Ser Ile Leu Gln Ser Arg Leu Ser Phe Phe Thr Pro Tyr
40 45 50

agc gcc gct gcc ctt ctc gtt gat ttc ctg ctc aat gta cta gct atc 303
Ser Ala Ala Ala Leu Leu Val Asp Phe Leu Leu Asn Val Leu Ala Ile
55 60 65

ttg ttc gca acc act tta tac tct tcg gcg cct ctt ctt ctc aat ctc 351
Leu Phe Ala Thr Thr Leu Tyr Ser Ser Ala Pro Leu Leu Leu Asn Leu
70 75 80

ctt cta ata tct ccc gct ctg ctg ata ctc ctc tct acg aaa cgt cct 399
Leu Leu Ile Ser Pro Ala Leu Leu Ile Leu Leu Ser Thr Lys Arg Pro
85 90 95

cgg acc ccc gtc aaa gcg aaa cct cct cgc cag tcc gct aga gct ggg 447
Arg Thr Pro Val Lys Ala Lys Pro Pro Arg Gln Ser Ala Arg Ala Gly
100 105 110 115

aaa gat gac tcg aaa cat gcg aca gcc ttg cca gag tct cta ccc att 495
Lys Asp Asp Ser Lys His Ala Thr Ala Leu Pro Glu Ser Leu Pro Ile
120 125 130

cat cca ttt ctc acg aca tat cgc gcc gcc atg atg gtt atc acg tgc 543
His Pro Phe Leu Thr Thr Tyr Arg Ala Ala Met Met Val Ile Thr Cys
135 140 145

atc gct atc ttg gct gtg gat ttt cgc att ttt cct cgc cga ttc gcc 591
Ile Ala Ile Leu Ala Val Asp Phe Arg Ile Phe Pro Arg Arg Phe Ala
150 155 160

aag gta gaa aac tgg ggt aca tca ctc atg gat ctg ggc gtt gga tcg 639
Lys Val Glu Asn Trp Gly Thr Ser Leu Met Asp Leu Gly Val Gly Ser
165 170 175

ttt gtc ttt tcg ggc gga gta gta tcc gct cgc tca cta ctc aag agc 687
Phe Val Phe Ser Gly Gly Val Val Ser Ala Arg Ser Leu Leu Lys Ser
180 185 190 195

agg acc aat ggc tct aaa agg ttg cct ctt gcc aag agg ttg att gcg 735
Arg Thr Asn Gly Ser Lys Arg Leu Pro Leu Ala Lys Arg Leu Ile Ala
200 205 210

tcg acg cga cac tct att cct ctg ctc gtc ctc ggc ctg att cgg cta 783
Ser Thr Arg His Ser Ile Pro Leu Leu Val Leu Gly Leu Ile Arg Leu
215 220 225

tac agc gtc aaa ggc ttg gac tat gcg gag cac gtc acc gag tac ggc 831
Tyr Ser Val Lys Gly Leu Asp Tyr Ala Glu His Val Thr Glu Tyr Gly

230 235 240

gta cat tgg aac ttc ttc ttt aca ttg ggt ctt ttg cct ccg ttc gtg 879
Val His Trp Asn Phe Phe Phe Thr Leu Gly Leu Leu Pro Pro Phe Val
245 250 255

gag gtc ttc gac gcc ttg gct acg atc att ccg tca tac gag gtt ctc 927
Glu Val Phe Asp Ala Leu Ala Thr Ile Ile Pro Ser Tyr Glu Val Leu
260 265 270 275

tcc gtg ggg atc gcc gtc ttg tat caa gtt gcc cta gag tca aca gac 975
Ser Val Gly Ile Ala Val Leu Tyr Gln Val Ala Leu Glu Ser Thr Asp
280 285 290

ttg aaa agc tac atc ctc gtc tcc cct cgt ggg cca agc tta ctg tcc 1023
Leu Lys Ser Tyr Ile Leu Val Ser Pro Arg Gly Pro Ser Leu Leu Ser
295 300 305

aag aat cgt gaa ggc gtc ttc tcc ttc tca ggt tat ctc gcg att ttt 1071
Lys Asn Arg Glu Gly Val Phe Ser Phe Ser Gly Tyr Leu Ala Ile Phe
310 315 320

ctt gct ggt cgt gcg atc ggc att cgg ata atc cct cgc gga act tct 1119
Leu Ala Gly Arg Ala Ile Gly Ile Arg Ile Ile Pro Arg Gly Thr Ser
325 330 335

ttc tca aga agc cca gaa cag gcc agg aga cgg gtc ctg atc agc ctt 1167
Phe Ser Arg Ser Pro Glu Gln Ala Arg Arg Arg Val Leu Ile Ser Leu
340 345 350 355

ggc gtg caa gcg tta gtg tgg acc act ctt ttt gtg ttg aac tcc act 1215
Gly Val Gln Ala Leu Val Trp Thr Thr Leu Phe Val Leu Asn Ser Thr
360 365 370

tat gcg atg gga tac gga gct aat atc cct gtc tcc cgc cgc ctc gct 1263
Tyr Ala Met Gly Tyr Gly Ala Asn Ile Pro Val Ser Arg Arg Leu Ala
375 380 385

aac atg ccc tat gtc ctt tgg gtt tcg gcg ttc aac acc gcg caa ctg 1311
Asn Met Pro Tyr Val Leu Trp Val Ser Ala Phe Asn Thr Ala Gln Leu
390 395 400

ttt gtg ttc tgc ctg atc gaa aca ctc tgc ttt cct gca gtt cat cgg 1359
Phe Val Phe Cys Leu Ile Glu Thr Leu Cys Phe Pro Ala Val His Arg
405 410 415

aca acg act caa gag agc gaa tct gag cga gtc gat ttt gct acg agc 1407
Thr Thr Thr Gln Glu Ser Glu Ser Glu Arg Val Asp Phe Ala Thr Ser
420 425 430 435

cga atc atg tcg gcc ttc aat aag aac agt ctc gcg atc ttt ctt ttg 1455
Arg Ile Met Ser Ala Phe Asn Lys Asn Ser Leu Ala Ile Phe Leu Leu

440 445 450

gcc aat ctt ctg act gga gct gtg aat ctg agc atc tcc aca att gat 1503
Ala Asn Leu Leu Thr Gly Ala Val Asn Leu Ser Ile Ser Thr Ile Asp
455 460 465

gct aat aca gcg cag gcc atc gct gtt ctc att gga tat tca tcc att 1551
Ala Asn Thr Ala Gln Ala Ile Ala Val Leu Ile Gly Tyr Ser Ser Ile
470 475 480

atc aca ggg gtt gct cta gca ttg cat cat gcc aat atc aaa gta ctt 1599
Ile Thr Gly Val Ala Leu Ala Leu His His Ala Asn Ile Lys Val Leu
485 490 495

cct ttc tag ggtatttacg agcaattggt ggtgtgttga agatatatag 1648
Pro Phe
500

<210> 42
<211> 27
<212> DNA
<213> Artificial

<400> 42
gccataataa gctaccgaat tgcaatg 27

<210> 43

<211> 26

<212> DNA

<213> Artificial

<400> 43

cattaacacc cccattgaca accacg 26

<210> 44

<211> 1869

<212> DNA

<213> Cryptococcus neoformans

<400> 44

ggggattaca agtcggccaa agaggccttt gtctcggata acccaggtgc ttctatctgg 60

agtatcaacg ctgtcagcct ggtcgcactg gtatgtagct cgttctccga ggggttctgt 120

catttggaga cgcttattaa ttgggatcgc aggcgacata tgctctctgg atcgccttat 180

cgccgtacat ccgtcatgga ctcctgaaca actacctgat ctgtgttctt cccctattat 240

tcggggtgac catcttctca acttcgcctc tcgtatttac ctcttttttg tccattattt 300

ccctcgcttt catcacgaaa tcccaaaaat gcttcaaatc tgtcagttcg cccgaaaagc 360

caaaaggcca atggctagac gaatcagact ccgatgagga accagcggaa cctgcttctg 420

cagctggatc tgcagcagtc tcaccagtaa agcttctacc ttcccaagtg gcgttcgctt 480

cgggatccct attatctccc gatccgacaa catcccccat gtcgccaagt agttcttcag 540

cttcaggaca tgaagaccct ttggggatta tgggcgttaa cagacggagg tcgctattag 600
aaggagtttc gcttgatgtt ccgtcacata tcgactccaa ggtcagaata tctcctgttc 660
cctacttgag gctcaaaaag tctagggcaa cgaaggcgca atgggtgaaa gaaaagggaa 720
gattaccatt tttgacagtg taccgagcgc acatgatgct catgactgtt atctgcatct 780
tggcggtaga ttttgaagtg tttcctagat ggcagggcaa gtgcgaagat tttggtacta 840
gtctggtaag ctttccttca gccatggtcc agtgctcacc gctctacttg ccgtagatgg 900
acgtgggtgt cgggtcattc gtcttttccc tcggtctcgt ctccacaaaa tctctttctc 960
ctccacctcc aactcctacg ccctcctcgc ccgctctcaa ctctcacatc attcccctca 1020
ccccgtcccc gttcacttcc atcctcatct cgctccgaaa atccatcccc atcctcgtcc 1080
tcggctttat acggttgatt atggtcaagg gatctgatta tcctgagcat gtgacggagt 1140
acggcgtgca ctggaatttc ttcttcaccc tcgcattggt tcctgtgctc gccgtgggca 1200
ttcgaccatt gacgcagtgg cttcgctgga gtgtgcttgg ggtaatcatc tctttgctgc 1260
atcagctgtg gttaacatat tatctccaat ccatcgtctt ctcattcggc cggtcaggta 1320
tctttctagc aaacaaggaa ggcttctcct ctcttcctgg ttatctttcc atatttttga 1380
tcggcttgtc tattggagat catgttttaa ggctcagttt accaccaaga agagagaggg 1440
tcgtgtcaga aacaaatgaa gagcatgagc agagtcattt tgagagaaaa aaattggatt 1500
tgattatgga gttgattgga tatagcttag gctggtgggc actcttagga ggctggattt 1560
gggccggcgg ggaggtatcc aggcgtttag taagtggaca tctttggtaa tattgtacct 1620
atactaatcc ctgcataaag gccaacgctc cttatgtatt ttgggtagcg gcatacaata 1680
ccacctttct cctcggctac ctcctcctta cccacattat tccatctccc acctcttccc 1740
aaacatcacc atcgatctta gtgcctccct tgctcgacgc tatgaataaa aacggtctcg 1800
cgatattttt ggcggccaac ttgcttacag gactggtgaa tgtgagcatg aagacaatgt 1860
atgcgccgg 1869

<210> 45

<211> 27

<212> DNA

<213> Artificial

<400> 45

gtaaaggaag gcgctagaaa agatatg 27

<210> 46

<211> 26

<212> DNA

<213> Artificial

<400> 46

ctcatcggag tctgattcgt ctagcc 26

<210> 47

<211> 470

<212> DNA

<213> Cryptococcus neoformans

<400> 47

gaaggcgcta gaaaagatat ggtcttgtca tagcattaaa tccccgccat aataagctac 60

tgaattgcaa tgggggatta caagtcggcc aaagaggcct ttgtctcgga taacccaggt 120

gcttctatct ggagtatcaa cgctgtcagc ctggtcgcac tggtatgtag ctcgttctcc 180

gaggggttct gtcatttgga gacgcttatt aattgggatc gcaggcgaca tatgctctct 240

ggatcgcctt atcgccgtac atccgtcatg gactcctgaa caactacctg atctgtgttc 300

ttcccctatt attcggggtg accatcttct caacttcgcc tctcgtattt acctcttttt 360

tgtccattat ttccctcgct ttcatcacga aatcccaaaa atgcttcaaa tctgtcagtt 420

cgcccgaaaa gccaaaaggc caatggctag acgaatcaga ctccgatgag 470

<210> 48

<211> 37

<212> DNA

<213> Artificial

<400> 48

gcccacgcgt cgactagtac tttttttttt ttttttt 37

<210> 49

<211> 29

<212> DNA

<213> Artificial

<400> 49

catcttggcg gtagattttg aagtgttcc 26

<210> 50

<211> 20

<212> DNA

<213> Artificial

<400> 50

ggccacgcgt cgactagtac 20

<210> 51

<211> 1136

<212> DNA

<213> Cryptococcus neoformans

<400> 51

gcggtagatt ttgaagtgtt ccctagatgg cagggcaagt gcgaagattt tggtactagt 60

ctgatggacg tgggtgtcgg gtcattcgtc ttttccctcg gtctcgtctc cacaaaatct 120

ctttctcctc cacctccaac tcctacgccc tcctcgcccg ctctcaactc tcacatcatt 180

cccctcaccc cgtccccgtt cacttccatc ctcatctcgc tccgaaaatc catccccatc 240

ctcgtcctcg gctttatacg gttgattatg gtcaagggat ctgattatcc tgagcatgtg 300

acggagtacg gcgtgcactg gaatttcttc ttcaccctcg cattggttcc tgtgctcgcc 360

gtgggcattc gaccattgac gcagtggctt cgctggagtg tgcttggggt aatcatctct 420

ttgctgcatc agctgtggtt aacatattat ctccaatcca tcgtcttctc attcggccgg 480

tcaggtatct ttctagcaaa caaggaaggc ttctcctctc ttcctggtta tctttccata 540

tttttgatcg gcttgtctat tggagatcat gttttaaggc tcagtttacc accaagaaga 600

gagagggtcg tgtcagaaac aaatgaagag catgagcaga gtcattttga gagaaaaaaa 660

ttggatttga ttatggagtt gattggatat agcttaggct ggtgggcact cttaggaggc 720

tggatttggg ccggcgggga ggtatccagg cgtttagcca acgctcctta tgtattttgg 780

gtagcggcat acaataccac ctttctcctc ggctacctcc tccttaccca cattattcca 840

tctcccacct cttcccaaac atcaccatcg atcttagtgc ctcccttgct cgacgctatg 900

aataaaaacg gtctcgcgat atttttggcg gccaacttgc ttacaggact ggtgaatgtg 960

agcatgaaga caatgtatgc gccggcgtgg ttgtcaatgg gggtgttaat gttgtatacc 1020

ttgacaatca gttgtgtagg gtggatactg aaaggacgga ggatcaagat atagttaaag 1080

tgtttaccat gcaggatact gagtatctcg gttcaaaaaa aaaaaaaaaa aaaaaa 1136

<210> 52

<211> 27

<212> DNA

<213> Artificial

<400> 52

gtcttgtcat agcattaaat ccccgcc 27

<210> 53

<211> 28

<212> DNA

<213> Artificial

<400> 53

gaaccgagat actcagtatc ctgcatgg 28

<210> 54

<211> 2045

<212> DNA

<213> Cryptococcus neoformans

<220>

<221> intron

<222> (137)..(198)

<220>

<221> intron

<222> (892)..(942)

<220>

<221> intron

<222> (1636)..(1686)

<220>

<221> CDS

<222> (44)..(2001)

<400> 54

gtcatagcat taaatccccg ccataataag ctactgaatt gca atg ggg gat tac 55

Met Gly Asp Tyr

1

aag tcg gcc aaa gag gcc ttt gtc tcg gat aac cca ggt gct tct atc 103
Lys Ser Ala Lys Glu Ala Phe Val Ser Asp Asn Pro Gly Ala Ser Ile
5 10 15 20

tgg agt atc aac gct gtc agc ctg gtc gca ctg gtatgtagct cgttctccga 156
Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu
25 30

ggggttctgt catttggaga cgcttattaa ttgggatcgc ag gcg aca tat gct 210
Ala Thr Tyr Ala
35

ctc tgg atc gcc tta tcg ccg tac atc cgt cat gga ctc ctg aac aac 258
Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu Leu Asn Asn
40 45 50

tac ctg atc tgt gtt ctt ccc cta tta ttc ggg gtg acc atc ttc tca 306
Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr Ile Phe Ser
55 60 65

act tcg cct ctc gta ttt acc tct ttt ttg tcc att att tcc ctc gct 354
Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile Ser Leu Ala
70 75 80

ttc atc acg aaa tcc caa aaa tgc ttc aaa tct gtc agt tcg ccc gaa 402
Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser Ser Pro Glu
85 90 95

aag cca aaa ggc caa tgg cta gac gaa tca gac tcc gat gag gaa cca 450
Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp Glu Glu Pro
100 105 110 115

gcg gaa cct gct tct gca gct gga tct gca gca gtc tca cca gta aag 498
Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser Pro Val Lys

```
                120                 125                 130
ctt cta cct tcc caa gtg gcg ttc gct tcg gga tcc cta tta tct ccc    546
Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu Leu Ser Pro
            135                 140                 145
gat ccg aca aca tcc ccc atg tcg cca agt agt tct tca gct tca gga    594
Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser Ala Ser Gly
        150                 155                 160
cat gaa gac cct ttg ggg att atg ggc gtt aac aga cgg agg tcg cta    642
His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg Arg Ser Leu
    165                 170                 175
tta gaa gga gtt tcg ctt gat gtt ccg tca cat atc gac tcc aag gtc    690
Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp Ser Lys Val
180                 185                 190                 195
aga ata tct cct gtt ccc tac ttg agg ctc aaa aag tct agg gca acg    738
Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser Arg Ala Thr
                200                 205                 210
aag gcg caa tgg gtg aaa gaa aag gga aga tta cca ttt ttg aca gtg    786
Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe Leu Thr Val
            215                 220                 225
tac cga gcg cac atg atg ctc atg act gtt atc tgc atc ttg gcg gta    834
Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile Leu Ala Val
        230                 235                 240
gat ttt gaa gtg ttt cct aga tgg cag ggc aag tgc gaa gat ttt ggt    882
Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu Asp Phe Gly
    245                 250                 255
act agt ctg gtaagctttc cttcagccat ggtccagtgc tcaccgctct            931
```

Thr Ser Leu

260

acttgccgta g atg gac gtg ggt gtc ggg tca ttc gtc ttt tcc ctc ggt 981

Met Asp Val Gly Val Gly Ser Phe Val Phe Ser Leu Gly

265 270 275

ctc gtc tcc aca aaa tct ctt tct cct cca cct cca act cct acg ccc 1029

Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr Pro Thr Pro

280 285 290

tcc tcg ccc gct ctc aac tct cac atc att ccc ctc acc ccg tcc ccg 1077

Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr Pro Ser Pro

295 300 305

ttc act tcc atc ctc atc tcg ctc cga aaa tcc atc ccc atc ctc gtc 1125

Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro Ile Leu Val

310 315 320

ctc ggc ttt ata cgg ttg att atg gtc aag gga tct gat tat cct gag 1173

Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp Tyr Pro Glu

325 330 335

cat gtg acg gag tac ggc gtg cac tgg aat ttc ttc ttc acc ctc gca 1221

His Val Thr Glu Tyr Gly Val His Trp Asn Phe Phe Phe Thr Leu Ala

340 345 350 355

ttg gtt cct gtg ctc gcc gtg ggc att cga cca ttg acg cag tgg ctt 1269

Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr Gln Trp Leu

360 365 370

cgc tgg agt gtg ctt ggg gta atc atc tct ttg ctg cat cag ctg tgg 1317

Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His Gln Leu Trp

375 380 385

tta aca tat tat ctc caa tcc atc gtc ttc tca ttc ggc cgg tca ggt 1365
Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly Arg Ser Gly
390 395 400

atc ttt cta gca aac aag gaa ggc ttc tcc tct ctt cct ggt tat ctt 1413
Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro Gly Tyr Leu
405 410 415

tcc ata ttt ttg atc ggc ttg tct att gga gat cat gtt tta agg ctc 1461
Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val Leu Arg Leu
420 425 430 435

agt tta cca cca aga aga gag agg gtc gtg tca gaa aca aat gaa gag 1509
Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr Asn Glu Glu
440 445 450

cat gag cag agt cat ttt gag aga aaa aaa ttg gat ttg att atg gag 1557
His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu Ile Met Glu
455 460 465

ttg att gga tat agc tta ggc tgg tgg gca ctc tta gga ggc tgg att 1605
Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly Gly Trp Ile
470 475 480

tgg gcc ggc ggg gag gta tcc agg cgt tta gtaagtggac atctttggta 1655
Trp Ala Gly Gly Glu Val Ser Arg Arg Leu
485 490

atattgtacc tatactaatc cctgcataaa g gcc aac gct cct tat gta ttt 1707
Ala Asn Ala Pro Tyr Val Phe
495 500

tgg gta gcg gca tac aat acc acc ttt ctc ctc ggc tac ctc ctc ctt 1755
Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly Tyr Leu Leu Leu

505 510 515

acc cac att att cca tct ccc acc tct tcc caa aca tca cca tcg atc 1803

Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr Ser Pro Ser Ile

520 525 530

tta gtg cct ccc ttg ctc gac gct atg aat aaa aac ggt ctc gcg ata 1851

Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn Gly Leu Ala Ile

535 540 545

ttt ttg gcg gcc aac ttg ctt aca gga ctg gtg aat gtg agc atg aag 1899

Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn Val Ser Met Lys

550 555 560

aca atg tat gcg ccg gcg tgg ttg tca atg ggg gtg tta atg ttg tat 1947

Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val Leu Met Leu Tyr

565 570 575 580

acc ttg aca atc agt tgt gta ggg tgg ata ctg aaa gga cgg agg atc 1995

Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys Gly Arg Arg Ile

585 590 595

aag ata tagttaaagt gtttaccatg caggatactg agtatctcgg ttca 2045

Lys Ile

<210> 55

<211> 23

<212> DNA

<213> Artificial

<400> 55

cagcctggtc gcactggcga cat 23

<210> 56

<211> 25

<212> DNA

<213> Artificial

<400> 56

cataaggagc gttggctaaa cgcct 25

<210> 57

<211> 1418

<212> DNA

<213> Cryptococcus neoformans

<400> 57

cagcctggtc gcactggcga catatgctct ctggatcgcc ttatcgccgt acatccgtca 60

tggactcctg aacaactacc tgatctgtgt tcttccccta ttattcgggg tgaccatctt 120

ctcaacttcg cctctcgtat ttacctcttt tttgtccatt atttccctcg ctttcatcac 180

gaaatcccaa aaatgcttca aatctgtcag ttcgcccgaa aagccaaaag gccaatggct 240

agacgaatca gactccgatg aggaaccagc ggaacctgct tctgcagctg gatctgcagc 300

agtctcacca gtaaagcttc taccttccca agtggcgttc gcttcgggat ccctattatc 360

tcccgatccg acaacatccc ccatgtcgcc aagtagttct tcagcttcag gacatgaaga 420

ccctttgggg attatgggcg ttaacagacg gaggtcgcta ttagaaggag tttcgcttga 480

tgttccgtca catatcgact ccaaggtcag aatatctcct gttccctact tgaggctcaa 540
aaagtctagg gcaacgaagg cgcaatgggt gaaagaaaag ggaagattac catttttgac 600
agtgtaccga gcgcacatga tgctcatgac tgttatctgc atcttggcgg tagattttga 660
agtgtttcct agatggcagg gcaagtgcga agattttggt actagtctga tggacgtggg 720
tgtcgggtca ttcgtctttt ccctcggtct cgtctccaca aaatctcttt ctcctccacc 780
tccaactcct acgccctcct cgcccgctct caactctcac atcattcccc tcaccccgtc 840
cccgttcact tccatcctca tctcgctccg aaaatccatc cccatcctcg tcctcggctt 900
tatacggttg attatggtca agggatctga ttatcctgag catgtgacgg agtacggcgt 960
gcactggaat ttcttcttca ccctcgcatt ggttcctgtg ctcgccgtgg gcattcgacc 1020
attgacgcag tggcttcgct ggagtgtgct tggggtaatc atctctttgc tgcatcagct 1080
gtggttaaca tattatctcc aatccatcgt cttctcattc ggccggtcag gtatctttct 1140
agcaaacaag gaaggcttct cctctcttcc tggttatctt tccatatttt tgatcggctt 1200
gtctattgga gatcatgttt taaggctcag tttaccacca agaagagaga gggtcgtgtc 1260
agaaacaaat gaagagcatg agcagagtca ttttgagaga aaaaaattgg atttgattat 1320
ggagttgatt ggatatagct taggctggtg ggcactctta ggaggctgga tttgggccgg 1380
cggggaggta tccaggcgtt tagccaacgc tccttatg 1418

<210> 58

<211> 1797

<212> DNA

<213> Cryptococcus neoformans

<220>

<221> CDS

<222> (1)..(1794)

<400> 58

atg ggg gat tac aag tcg gcc aaa gag gcc ttt gtc tcg gat aac cca 48
Met Gly Asp Tyr Lys Ser Ala Lys Glu Ala Phe Val Ser Asp Asn Pro
1 5 10 15

ggt gct tct atc tgg agt atc aac gct gtc agc ctg gtc gca ctg gcg 96
Gly Ala Ser Ile Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu Ala
20 25 30

aca tat gct ctc tgg atc gcc tta tcg ccg tac atc cgt cat gga ctc 144
Thr Tyr Ala Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu
35 40 45

ctg aac aac tac ctg atc tgt gtt ctt ccc cta tta ttc ggg gtg acc 192
Leu Asn Asn Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr
50 55 60

atc ttc tca act tcg cct ctc gta ttt acc tct ttt ttg tcc att att 240
Ile Phe Ser Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile
65 70 75 80

tcc ctc gct ttc atc acg aaa tcc caa aaa tgc ttc aaa tct gtc agt 288
Ser Leu Ala Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser
85 90 95

tcg ccc gaa aag cca aaa ggc caa tgg cta gac gaa tca gac tcc gat 336
Ser Pro Glu Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp
100 105 110

gag gaa cca gcg gaa cct gct tct gca gct gga tct gca gca gtc tca 384
Glu Glu Pro Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser
115 120 125

cca gta aag ctt cta cct tcc caa gtg gcg ttc gct tcg gga tcc cta 432
Pro Val Lys Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu
130 135 140

tta tct ccc gat ccg aca aca tcc ccc atg tcg cca agt agt tct tca 480
Leu Ser Pro Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser
145 150 155 160

gct tca gga cat gaa gac cct ttg ggg att atg ggc gtt aac aga cgg 528
Ala Ser Gly His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg
165 170 175

agg tcg cta tta gaa gga gtt tcg ctt gat gtt ccg tca cat atc gac 576
Arg Ser Leu Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp
180 185 190

tcc aag gtc aga ata tct cct gtt ccc tac ttg agg ctc aaa aag tct 624
Ser Lys Val Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser
195 200 205

agg gca acg aag gcg caa tgg gtg aaa gaa aag gga aga tta cca ttt 672
Arg Ala Thr Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe
210 215 220

ttg aca gtg tac cga gcg cac atg atg ctc atg act gtt atc tgc atc 720
Leu Thr Val Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile
225 230 235 240

ttg gcg gta gat ttt gaa gtg ttt cct aga tgg cag ggc aag tgc gaa 768
Leu Ala Val Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu
245 250 255

gat ttt ggt act agt ctg atg gac gtg ggt gtc ggg tca ttc gtc ttt 816
Asp Phe Gly Thr Ser Leu Met Asp Val Gly Val Gly Ser Phe Val Phe

260 265 270

tcc ctc ggt ctc gtc tcc aca aaa tct ctt tct cct cca cct cca act 864

Ser Leu Gly Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr

275 280 285

cct acg ccc tcc tcg ccc gct ctc aac tct cac atc att ccc ctc acc 912

Pro Thr Pro Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr

290 295 300

ccg tcc ccg ttc act tcc atc ctc atc tcg ctc cga aaa tcc atc ccc 960

Pro Ser Pro Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro

305 310 315 320

atc ctc gtc ctc ggc ttt ata cgg ttg att atg gtc aag gga tct gat 1008

Ile Leu Val Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp

325 330 335

tat cct gag cat gtg acg gag tac ggc gtg cac tgg aat ttc ttc ttc 1056

Tyr Pro Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe Phe Phe

340 345 350

acc ctc gca ttg gtt cct gtg ctc gcc gtg ggc att cga cca ttg acg 1104

Thr Leu Ala Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr

355 360 365

cag tgg ctt cgc tgg agt gtg ctt ggg gta atc atc tct ttg ctg cat 1152

Gln Trp Leu Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His

370 375 380

cag ctg tgg tta aca tat tat ctc caa tcc atc gtc ttc tca ttc ggc 1200

Gln Leu Trp Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly

385 390 395 400

cgg tca ggt atc ttt cta gca aac aag gaa ggc ttc tcc tct ctt cct 1248

Arg Ser Gly Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro
405 410 415

ggt tat ctt tcc ata ttt ttg atc ggc ttg tct att gga gat cat gtt 1296
Gly Tyr Leu Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val
420 425 430

tta agg ctc agt tta cca cca aga aga gag agg gtc gtg tca gaa aca 1344
Leu Arg Leu Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr
435 440 445

aat gaa gag cat gag cag agt cat ttt gag aga aaa aaa ttg gat ttg 1392
Asn Glu Glu His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu
450 455 460

att atg gag ttg att gga tat agc tta ggc tgg tgg gca ctc tta gga 1440
Ile Met Glu Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly
465 470 475 480

ggc tgg att tgg gcc ggc ggg gag gta tcc agg cgt tta gcc aac gct 1488
Gly Trp Ile Trp Ala Gly Gly Glu Val Ser Arg Arg Leu Ala Asn Ala
485 490 495

cct tat gta ttt tgg gta gcg gca tac aat acc acc ttt ctc ctc ggc 1536
Pro Tyr Val Phe Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly
500 505 510

tac ctc ctc ctt acc cac att att cca tct ccc acc tct tcc caa aca 1584
Tyr Leu Leu Leu Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr
515 520 525

tca cca tcg atc tta gtg cct ccc ttg ctc gac gct atg aat aaa aac 1632
Ser Pro Ser Ile Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn
530 535 540

ggt ctc gcg ata ttt ttg gcg gcc aac ttg ctt aca gga ctg gtg aat 1680

Gly Leu Ala Ile Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn

545 550 555 560

gtg agc atg aag aca atg tat gcg ccg gcg tgg ttg tca atg ggg gtg 1728

Val Ser Met Lys Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val

565 570 575

tta atg ttg tat acc ttg aca atc agt tgt gta ggg tgg ata ctg aaa 1776

Leu Met Leu Tyr Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys

580 585 590

gga cgg agg atc aag ata tag 1797

Gly Arg Arg Ile Lys Ile

595

<210> 59

<211> 598

<212> PRT

<213> Cryptococcus neoformans

<400> 59

Met Gly Asp Tyr Lys Ser Ala Lys Glu Ala Phe Val Ser Asp Asn Pro

1 5 10 15

Gly Ala Ser Ile Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu Ala

20 25 30

Thr Tyr Ala Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu

35 40 45

Leu Asn Asn Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr
50 55 60
Ile Phe Ser Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile
65 70 75 80
Ser Leu Ala Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser
85 90 95
Ser Pro Glu Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp
100 105 110
Glu Glu Pro Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser
115 120 125
Pro Val Lys Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu
130 135 140
Leu Ser Pro Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser
145 150 155 160
Ala Ser Gly His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg
165 170 175
Arg Ser Leu Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp
180 185 190
Ser Lys Val Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser
195 200 205
Arg Ala Thr Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe
210 215 220
Leu Thr Val Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile
225 230 235 240
Leu Ala Val Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu
245 250 255

Asp Phe Gly Thr Ser Leu Met Asp Val Gly Val Gly Ser Phe Val Phe

260 265 270

Ser Leu Gly Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr

275 280 285

Pro Thr Pro Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr

290 295 300

Pro Ser Pro Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro

305 310 315 320

Ile Leu Val Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp

325 330 335

Tyr Pro Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe Phe Phe

340 345 350

Thr Leu Ala Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr

355 360 365

Gln Trp Leu Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His

370 375 380

Gln Leu Trp Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly

385 390 395 400

Arg Ser Gly Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro

405 410 415

Gly Tyr Leu Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val

420 425 430

Leu Arg Leu Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr

435 440 445

Asn Glu Glu His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu

450 455 460

Ile Met Glu Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly

465 470 475 480

Gly Trp Ile Trp Ala Gly Gly Glu Val Ser Arg Arg Leu Ala Asn Ala

485 490 495

Pro Tyr Val Phe Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly

500 505 510

Tyr Leu Leu Leu Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr

515 520 525

Ser Pro Ser Ile Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn

530 535 540

Gly Leu Ala Ile Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn

545 550 555 560

Val Ser Met Lys Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val

565 570 575

Leu Met Leu Tyr Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys

580 585 590

Gly Arg Arg Ile Lys Ile

595

<210> 60

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially
synthesized primer sequence

<400> 60
aaagaattca tggcaacagt acatcagaag 30

<210> 61
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Description of Artificial Sequence:an artificially
synthesized primer sequence

<400> 61
gggcactgtt gaaaaaccta 20

<210> 62
<211> 1428
<212> DNA
<213> Saccharomyces cerevisiae
<220>
<221> promoter

<222> (1)..(1428)

<400> 62

```
gttgttcaaa atgggggtaa aattgagacg tcttacttga gcggcattta cgatcattct   60
tattacatca ttccaagtaa taaagctctt gactccttca atgatttacc tgagattata  120
gatgataatg atggtatagt tacagaattt ttcattgaac gctgcttgta ttatcaaaaa  180
ttactacacc caatagattt atggtcaaaa cccttcctca gcacaataga gtttcaagtt  240
tcgtcttctt caaagttatt gcatcatgaa ttttcttctt ccccttttct gaatgttact  300
atcactggat tctctggcgt agagctgtta catctgacta aagtattaaa tcttctaaaa  360
ccaatgggca tcaattatgt agaatacctc aataaatcca ctgacattct gctaatcaac  420
ttagcagctt tacccagtat cccgaaaacc catccgttat ggtcgaatga atttagcgat  480
ctttttactc agttttgcat taataacaat aatgatgatc ctggtgataa taacagaaaa  540
gattttcaaa ataattcaat cttgagaaat tcgatgaaaa ggaaaattga atatatcaag  600
aaattccact ccataccggt agttactcca gcatttattt ttaaattatt gtccgctgca  660
tctggagaaa ataatgaaat ctttttaaac aatatcaagt ggtgtattat ctgcccaaga  720
ggacacaagg acgattttaa atgtaagata aaaaaaccat actataccag cattagttca  780
gaaaaaaagt accaaaacaa tgatccaaaa atcgacaaaa ctattctttt gaaaagaaac  840
aattcctcat tatcggagca ctctatgaaa gataccaaaa acgaattatt gcagaaaatt  900
agagaaactg attctggaag aaaaaagcgt agtgtctcat cgagtatcat ggatgtttct  960
tcagagagac aaatgccgga tacgaaaagg atcaagttgg agtcactgcc aaaaaatttc 1020
gttcctaaac aaattaaacg aaccacgagt tggggcacaa taatgtcaga aaatgtgcct 1080
acagagcagc cgactgcaat ttctaatcca gaagagatcc caagaactga ggaagtttca 1140
catactcaag ttacctatgg ctccattcaa gataagaaac gtactgcctc tttagaaaaa 1200
cctatgagac gacagacaag aaatcagaca aaggaattag attcttgaaa tgtagtccgt 1260
aattttataa gatattcatt tacatacgcc atctacagca ttattcaaat ctactcatct 1320
atatgtatta ccgttttgta tgataatact ttccatgaca tgctcgcgtg aaaaaacagc 1380
atgagaaaaa gaggatcgca ataagaagac acgtaaatat ctaaataa               1428
```

<210> 63

<211> 133

<212> DNA

<213> Saccharomyces cerevisiae

<220>

<221> terminator

<222> (1)..(133)

<400> 63

taacacacca tccacatttc catgtagttc gtatacaaac cctaccagta aaataaaatt 60

aactcctatg tgctttaaat aaaaattata aaccgcctcc aatagttgac gtagtcaggc 120

atgaaagtgc tac 133

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/05899

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C12N15/09, C07K14/37, 16/14, G01N33/15, 33/50, A61K39/395, 45/00, 31/4355, 31/4365, 31/437, 31/44, 31/472, 31/4725, 31/5377, A61P31/10, C07D213/16, 213/61, 213/65, 213/69, 213/74, 217/18, 217/20, 401/10, 401/12, 405/06, 405/10, 405/12, 471/04, 491/048, 491/056, 495/04, 498/04, 513/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C12N15/09, C07K14/37, 16/14, G01N33/15, 33/50, A61K39/395, 45/00, 31/4355, 31/4365, 31/437, 31/44, 31/472, 31/4725, 31/5377, A61P31/10, C07D213/16, 213/61, 213/65, 213/69, 213/74, 217/18, 217/20, 401/10, 401/12, 405/06, 405/10, 405/12, 471/04, 491/048, 491/056, 495/04, 498/04, 513/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA (STN), REGISTRY (STN), MEDLINE (STN), Genbank/EMBL/DDBJ/PIR/SwissProt/Genseq, WPI (DIALOG), BIOSIS (DIALOG)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. Miosga et al., "Sequence Analysis of a 33.1 kb Fragment from the Left Arm of Saccharomyces cerevisiae Chromosome X, Including Putative Proteins with Leucine Zippers, a Fungal Zn(II)2-Cys6 Binuclear Cluster Domain and a Putative α2-SCB-α2 Binding Site", Yeast, Vol.11, pages 681 to 689, (1995), the whole document	1-11
X	US 4013666 A (G. D. Searle & Co.), 22 March, 1997 (22.03.97), Claims; working example, etc. (Family: none)	15-22
X	WO 00/01387 A1 (Celgro, a division of Celegene Corporation), 13 January, 2000 (13.01.00), Claims; working example, etc. & AU 9948491 A	15-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 September, 2001 (25.09.01)	02 October, 2001 (02.10.01)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/05899

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 23
   because they relate to subject matter not required to be searched by this Authority, namely:

   Claim 23 pertains to methods for treatment of the human or animal body by therapy and thus relates to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☒ Claims Nos.: 12-14
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   Although the statement in the description is taken into consideration, it is unknown what particular compounds are involved in the scope of the "compound having an antifungal effect" and the "antifungal agent" as described in the above claims. Thus, no international search can be practiced on the above claims.

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

国際調査報告

国際出願番号 PCT／JP01／05899

A. 発明の属する分野の分類（国際特許分類（IPC））

Int. Cl⁷ C12N15/09, C07K14/37, 16/14, G01N33/15, 33/50, A61K39/395, 45/00, 31/4355, 31/4365, 31/437, 31/44, 31/472, 31/4725, 31/5377, A61P31/10, C07D213/16, 213/61, 213/65, 213/69, 213/74, 217/18, 217/20, 401/10, 401/12, 405/06, 405/10, 405/12, 471/04, 491/048, 491/056, 495/04, 498/04, 513/04

B. 調査を行った分野

調査を行った最小限資料（国際特許分類（IPC））

Int. Cl⁷ C12N15/09, C07K14/37, 16/14, G01N33/15, 33/50, A61K39/395, 45/00, 31/4355, 31/4365, 31/437, 31/44, 31/472, 31/4725, 31/5377, A61P31/10, C07D213/16, 213/61, 213/65, 213/69, 213/74, 217/18, 217/20, 401/10, 401/12, 405/06, 405/10, 405/12, 471/04, 491/048, 491/056, 495/04, 498/04, 513/04

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

CA(STN), REGISTRY(STN), MEDLINE(STN), Genbank/EMBL/DDBJ/PIR/SwissProt/Genseq, WPI(DIALOG), BIOSIS(DIALOG)

C. 関連すると認められる文献

引用文献のカテゴリー＊	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
X	T. Miosga et al., "Sequence Analysis of a 33.1 kb Fragment from the Left Arm of Saccharomyces cerevisiae Chromosome X, Including Putative Proteins with Leucine Zippers, a Fungal Zn(II)2-Cys6 Binuclear Cluster Domain and a Putative α2-SCB-α2 Binding Site" Yeast, Vol. 11, p. 681-689 (1995), 文献全体参照	1-11
X	US 4013666 A, (G. D. Searle & Co.), 22. 3月. 1977 (22. 03. 97), 特許請求の範囲、実施例等参照，（ファミリーなし）	15-22
X	WO 00/01387 A1, (CELGRO, a division of CELEGENE CORPORATION), 13. 1月. 2000 (13. 01. 00), 特許請求の範囲、実施例等参照, & AU 9948491 A	15-22

☐ C欄の続きにも文献が列挙されている。　☐ パテントファミリーに関する別紙を参照。

＊ 引用文献のカテゴリー
「A」特に関連のある文献ではなく、一般的技術水準を示すもの
「E」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「L」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「O」口頭による開示、使用、展示等に言及する文献
「P」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「T」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「X」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Y」特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「&」同一パテントファミリー文献

国際調査を完了した日 25. 09. 01	国際調査報告の発送日 02.10.01
国際調査機関の名称及びあて先 日本国特許庁（ISA／JP） 郵便番号100−8915 東京都千代田区霞が関三丁目4番3号	特許庁審査官（権限のある職員） 4N 9451 坂崎 恵美子 電話番号 03−3581−1101 内線 3488

第Ⅰ欄 請求の範囲の一部の調査ができないときの意見（第１ページの２の続き）

法第８条第３項（ＰＣＴ１７条(2)(a)）の規定により、この国際調査報告は次の理由により請求の範囲の一部について作成しなかった。

1. ☒ 請求の範囲 ２３ は、この国際調査機関が調査をすることを要しない対象に係るものである。つまり、
請求項２３は治療による人体又は動物の体の処置方法に関するものであって、PCT17条(2)(a)(i)及びPCT規則39.1(iv)の規定によりこの国際調査機関が調査することを要しない対象に係るものである。

2. ☒ 請求の範囲 １２－１４ は、有意義な国際調査をすることができる程度まで所定の要件を満たしていない国際出願の部分に係るものである。つまり、
前記請求の範囲に記載の「抗真菌作用を有する化合物」及び「抗真菌剤」について、明細書の記載を参酌しても、具体的にはどのような化合物が包含され、どのような化合物が包含されないのかが全く不明であるから、前記請求の範囲の記載は著しく不明確である。したがって、前記請求の範囲については、有意義な国際調査をすることができない。

3. ☐ 請求の範囲 ＿＿＿ は、従属請求の範囲であってＰＣＴ規則6.4(a)の第２文及び第３文の規定に従って記載されていない。

第Ⅱ欄 発明の単一性が欠如しているときの意見（第１ページの３の続き）

次に述べるようにこの国際出願に二以上の発明があるとこの国際調査機関は認めた。

1. ☐ 出願人が必要な追加調査手数料をすべて期間内に納付したので、この国際調査報告は、すべての調査可能な請求の範囲について作成した。

2. ☐ 追加調査手数料を要求するまでもなく、すべての調査可能な請求の範囲について調査することができたので、追加調査手数料の納付を求めなかった。

3. ☐ 出願人が必要な追加調査手数料を一部のみしか期間内に納付しなかったので、この国際調査報告は、手数料の納付のあった次の請求の範囲のみについて作成した。

4. ☐ 出願人が必要な追加調査手数料を期間内に納付しなかったので、この国際調査報告は、請求の範囲の最初に記載されている発明に係る次の請求の範囲について作成した。

追加調査手数料の異議の申立てに関する注意

☐ 追加調査手数料の納付と共に出願人から異議申立てがあった。

☐ 追加調査手数料の納付と共に出願人から異議申立てがなかった。

ENGLISH TRANSLATION OF

PCT PUBLICATION: WO 02/04626 A1
Entitled: "FUNGAL CELL WALL SYNTHESIS GENE"

Cited in Information Disclosure Statement

Re: US Application Serial No.: 10/536,935
Int'l Filing Date: November 21, 2003

Attorney Docket No.: 082368-004400US

DESCRIPTION

FUNGAL CELL WALL SYNTHESIS GENE

Technical Field

The present invention relates to DNAs encoding proteins participating in fungal cell wall synthesis, proteins encoded by the DNAs, methods for examining whether or not a certain compound has an influence on the transport process involved in the transport of GPI-anchored proteins to the cell wall, and antifungal agents having an influence on the transport process involved in the transport of GPI-anchored proteins to the cell wall.

Background Art

In recent years, management of opportunistic infections are gaining importance more than ever due to an increase in the number of elderly people and immunocompromised patients as a result of advanced chemotherapies, etc. Deep-seated mycosis due to *Candida*, *Aspergillus*, *Cryptococcus*, and such, account for a portion of such opportunistic infections, and the proportion is increasing year after year. The fact that opportunistic infections by many avirulent bacteria occur one after another, shows that the problem of infectious diseases will not end as long as there are underlying diseases that diminish the immune functions of patients. Although new strategies for infectious diseases control, including the problem of resistant bacteria, will be one of the crucial issues in the soon-to-come aged society, extremely few effective therapeutic agents exist at present.

Up to now, therapeutic agents for fungal infections were developed based mainly on the strategy of creating novel compounds by chemically modifying known structure. However, due to problems such as the emergence of resistant bacteria, the development of new drugs based on new mechanisms is eagerly anticipated.

Considering such circumstances, the inventors focused on a novel

approach in the area of antifungal agents in which the variety of therapeutic agents is still insufficient. Namely, the present inventors concentrated on influencing the onset, progress, and persistence of infections by preventing pathogens from showing pathogenicity. In order to avoid the establishment and progress of infection, the inventors thought that the most effective way would be to inhibit the adhesion onto the host, which is the first step in the establishment of infection, and the subsequent progression of colonization. In addition, a new unprecedented approach, namely, the inhibition of the expression of adhesion factors themselves, was also carried out.

In order to inhibit the expression of adhesion factors, the present inventors directed their attention to the hypothesis that cell wall glycoproteins such as adhesion factors are first GPI (Glycosylphosphatidylinositol)-anchored to the cell membrane, and then transported to the cell wall (Fig. 1). To date, 30 or more cell wall glycoproteins including adhesion ligands have been found to be transported via GPI-anchoring (referred to as GPI-anchored proteins). Hence, it was thought that if this transport step is inhibited, it may be quite possible to inhibit the expression of adhesion factors and major cell wall-constituting proteins at the cell wall (Hamada K et al, Mol. Gen. Genet., 258: 53-59, 1998). GPI-anchored proteins have been reported to be present in *Candida,* which is a pathogenic fungi (Kapteyn JC et al, Eur. J. Cell Biol., 65:402-407, 1994).

The inventors initiated their research believing that novel antifungal agents that inhibit cell wall synthesis can be produced by inhibiting the process that transports GPI-anchored proteins existing in the cell membrane of a fungus to the cell wall.

Disclosure of the Invention

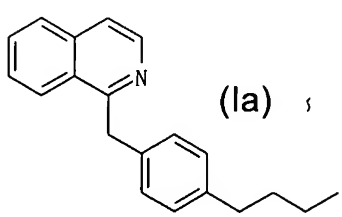
An objective of this invention is to develop antifungal agents showing effects against the onset, progress, and persistence of infections by inhibiting the expression of cell wall glycoproteins,

inhibiting the cell wall assembly and also adhesion onto cells, and preventing pathogens from showing pathogenicity.

In order to screen for compounds that inhibit the process that transports GPI-anchored proteins to the cell wall, the present inventors produced a reporter system that uses a fusion protein comprising a reporter enzyme and a transport signal existing in the C-terminus of one of the GPI-anchored proteins, CWP2 (Van Der Vaat JM et al, J. Bacteriol., 177:3104-3110,1995).

When a DNA comprising a secretion signal gene + reporter enzyme gene + CWP2 C-terminus gene (present or absent) was constructed, and the fusion protein was expressed in *Saccharomyces cerevisiae* (hereinafter, referred to as *S. cerevisiae*), it was demonstrated that activity of the reporter enzyme is detected in the cell wall when the CWP2 C-terminus is present, and in the culture supernatant when the CWP2 C-terminus is absent. Accordingly, it was predicted that if the process that transports GPI-anchored proteins to the cell wall is inhibited by a test sample, the activity of the reporter enzyme in the cell wall will be diminished, or the activity of the reporter enzyme will be found in the culture supernatant. Thus was initiated the screening for compounds that inhibit the process that transports GPI-anchored proteins to the cell wall using this reporter system.

From the screening using this reporter system, several compounds that inhibit the process that transports GPI-anchored proteins to the cell wall were discovered. A representative example is the compound shown in formula (Ia).



(Ia) ,

The compound shown in the aforementioned formula (Ia) (hereinafter abbreviated as "compound (Ia)") inhibits the growth of *S. cerevisiae* and *Candida albicans* (hereinafter, referred to as *C. albicans*), and *C.*

albicans cultured in the presence of the aforementioned compound (Ia) shows a weak ability to adhere onto cells. Thus, the aforementioned compound (Ia) was confirmed to suit the initial objectives of the invention, which was to find a compound that inhibits the adhesion of fungi, due to suppressing the expression of the fungal adhesins, based on the inhibition of transport system of GPI-anchored proteins to the cell wall. Furthermore, observations using a transmission electron microscope confirmed that *C. albicans* cultured in the presence of the aforementioned compound (Ia) has an abnormality in its cell wall synthesis.

Using the aforementioned compound (Ia), the present inventors proved that antifungal agents based on the mechanism that inhibits the process that transports GPI-anchored proteins to the cell wall, could be achieved.

Furthermore, to specify the target protein on which the aforementioned compound (Ia) acts, the present inventors searched for genes that confer resistance to the aforementioned compound (Ia).

A plasmid library of the *S. cerevisiae* gene was introduced into *S. cerevisiae*, and by overexpression, plasmids were collected that showed resistance to the abovementioned compound (Ia). The resistant gene was then cloned, the nucleotide sequence was determined, and the gene was named GWT1 (SEQ ID NO: 1). In *S. cerevisiae* overexpressing the GWT1 gene product, the aforementioned reporter enzyme that has the C-terminus of a GPI-anchored protein was transported to the cell wall, even in the presence of the aforementioned compound (Ia). Furthermore, observations under a transmission electron microscope confirmed that the cell wall is normal even in the presence of the aforementioned compound (Ia).

Moreover, when point mutations were randomly introduced to the genomic DNA of *S. cerevisiae*, and mutant strains R1 and R5 showing specific resistance to the aforementioned compound (Ia) were isolated, point mutations involving changes of the 405th codon of the GWT1 gene from GTC to ATC in the R1 mutant strain, and the 140th codon from GGG

to AGG in the R5 mutant strain were discovered. Since resistance to the aforementioned compound (Ia) was seen when these mutant GWT1 genes were introduced to a GWT1 gene-disrupted strain, resistance to this compound was found to be explainable by the GWT1 gene alone. Therefore, this suggested that the aforementioned compound (Ia) directly acts on the GWT1 gene product to inhibit the function of the GWT1 protein.

By similar methods, the resistant genes of *C. albicans* (SEQ ID NOS: 3 and 5) were cloned, the nucleotide sequences were determined, and the genes were named CaGWT1.

Furthermore, a database homology search using GWT1, revealed a homologue (SEQ ID NO: 27) of *Schizosaccharomyces pombe* (hereinafter, referred to as *S. pombe*). Furthermore, PCR with primers based on the sequence of the highly conserved region in the proteins encoded by the GWT1 genes of *S. cerevisiae*, *S. pombe*, and *C. albicans*, yielded homologues (SEQ ID NOS: 39 and 41) of *Aspergillus fumigatus* (hereinafter, referred to as *A. fumigatus*). Furthermore, by performing PCR based on the sequence discovered from a database homology search with GWT1, revealed homologues (SEQ ID NOS: 54 and 58) of *Cryptococcus neoformans* (hereinafter, referred to as *C. neoformans*).

More specifically, this invention relates to the following.

1. A DNA that encodes a protein having an activity to confer resistance to the compound shown in formula (Ia) on a fungus when the DNA is overexpressed in the fungus, wherein the DNA is selected from the group consisting of:

(a) A DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59.

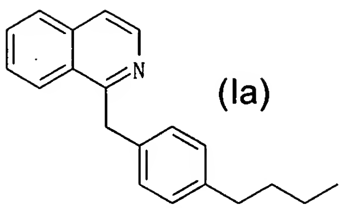
(b) A DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58.

(c) A DNA that hybridizes under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58.

(d) A DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59, wherein one or more amino

acids have been added, deleted, substituted, and/or inserted.

(e) A DNA that is amplified using SEQ ID NOS: 29 and 31 or SEQ ID NOS: 29 and 30 as primers.



(Ia)

2. A DNA that encodes a protein having an activity to decrease the amount of a GPI-anchored protein in the cell wall of a fungus due to a defect in the function of the DNA, wherein the DNA is selected from the group consisting of:

(a) A DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59,

(b) A DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58,

(c) A DNA that hybridizes under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58,

(d) A DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59, wherein one or more amino acids have been added, deleted, substituted, and/or inserted, and

(e) A DNA that is amplified using SEQ ID NOS: 29 and 31 or SEQ ID NOS: 29 and 30 as primers,

and wherein, "stringent conditions" refer to: for example, hybridization in 4x SSC at 65°C, then washing in 0.1x SSC for 1 hour at 65°C; or in a different method, "stringent conditions" are 4x SSC at 42°C in 50% formamide; or, hybridization in PerfectHyb™ (TOYOBO) solution for 2.5 hours at 65°C, then washing in (i) 2x SSC, 0.05% SDS solution at 25°C for 5 minutes, (ii) 2x SSC, 0.05% SDS solution at 25°C for 15 minutes, and (iii) 0.1x

SSC, 0.1% SDS solution at 50°C for 20 minutes;

a "defect in the DNA function" can occur, when the functional gene product of the DNA is not expressed or when the expression is diminished, for example by inserting a DNA that is irrelevant to the coding region of the DNA, for example a selection marker, using the homologous recombination technique;

and a decrease in the protein derived from the GPI-anchored protein in the fungal cell wall is quantified by using any one of the following methods alone or in combination: (i) a reporter system reflecting the process that transports GPI-anchored proteins to the cell wall, (ii) an ELISA that quantifies a GPI-anchored protein in the cell wall, (iii) measuring the activity of a GPI-anchored protein, such as adhesion onto animal cells, or (4) observing the flocculent, fibrous structure of the outermost layer of the fungal cell by a transmission electron microscope.

3. A protein encoded by the DNA of 1 or 2.

4. A vector into which the DNA of 1 or 2 has been inserted.

5. A transformant harboring the DNA of 1 or 2, or the vector of 4.

6. The transformant of 5 which is a fungus that overexpresses the protein of 3.

7. A fungus, wherein the function of the protein of 3 is defective.

8. A method for producing the protein of 3, which comprises the steps of culturing the transformant of 5, and collecting the expressed protein from the transformant, or from the culture supernatant thereof.

9. An antibody that binds to the protein of 3.

10. A method of screening for a compound having an antifungal action, wherein the method comprises the steps of:

(a) contacting a test sample with the protein of 3;

(b) detecting the binding activity between the protein and the test sample; and

(c) selecting a compound having an activity to bind to the protein.

11. A method of screening for a compound that has an antifungal action, which comprises the steps of:

(a) contacting a test sample with a fungus that is overexpressing the protein of 3;

(b) detecting the amount of transport of a GPI-anchored protein to the cell wall in the fungus; and

(c) selecting a compound that diminishes the amount of transport of the GPI-anchored protein to the cell wall detected in step (b) as compared to the amount of transport detected when the test sample was contacted with a fungus that is not overexpressing the protein of 3,

wherein, a decrease in the amount of GPI-anchored protein transported to the cell wall that results due to the test sample can be detected, for example, by detecting a decrease in growth rate, swelling, or temperature sensitivity of the cell, or by detecting a decrease of the protein derived from the GPI-anchored protein in the cell wall, but preferably, by detecting a decrease in the protein derived from the GPI-anchored protein at the cell wall;

and wherein a decrease of the protein derived from the GPI-anchored protein is quantified by using any one of the following methods alone or in combination:

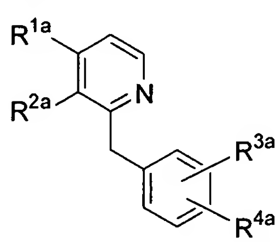
(i) a reporter system reflecting the process that transports GPI-anchored proteins to the cell wall, (ii) an ELISA that quantifies one type of the GPI-anchored protein in the cell wall, (iii) measuring the activity of a GPI-anchored protein such as adhesion to animal cells, and (iv) observing the flocculent, fibrous structure of the outermost layer of a fungal cell by a transmission electron microscope.

12. A compound having an antifungal action that is isolated by the screening of 10 or 11.

13. An antifungal agent, comprising as an active ingredient a compound that inhibits the transport of GPI-anchored proteins to the cell wall of a fungus.

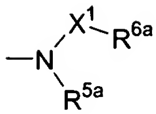
14. An antifungal agent, comprising as an active ingredient the antibody of 9 or the compound of 12.

15. The antifungal agent of 13, comprising as an active ingredient the compound represented by the general formula (I), a salt thereof, or a hydrate thereof, wherein in formula (I):



(I)

[R^{1a} and R^{2a} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro group, cyano group, trifluoromethyl group, trifluoromethoxy group, a substituted or unsubstituted C_{1-6} alkyl group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, a substituted or unsubstituted C_{1-6} alkoxy group, or a group represented by the formula:

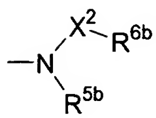


(wherein X^1 stands for a single bond, carbonyl group, or a group represented by the formula $-S(O)_2-$;

R^{5a} and R^{6a} are identical to or different from each other and denote a hydrogen atom or a substituted or unsubstituted C_{1-6} alkyl group). Furthermore, R^{1a} and R^{2a} may together form a condensed ring selected from the group consisting of a substituted or unsubstituted benzene ring, a substituted or unsubstituted pyridine ring, a substituted or unsubstituted pyrrole ring, a substituted or unsubstituted thiophene ring, a substituted or unsubstituted furan ring, a substituted or unsubstituted pyridazine ring, a substituted or unsubstituted pyrimidine ring, a substituted or unsubstituted pyrazine ring, a substituted or unsubstituted imidazole ring, a substituted or unsubstituted oxazole ring, a substituted or unsubstituted thiazole ring, a substituted or unsubstituted pyrazole ring, a substituted or unsubstituted isoxazole ring, a substituted or unsubstituted isothiazole ring, a substituted or unsubstituted cyclohexane ring, and a substituted or unsubstituted cyclopentane ring;

R^{3a} and R^{4a} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro group, cyano group, carboxyl group, formyl group, hydroxyimino group, trifluoromethyl group, trifluoromethoxy group, C_{1-6} alkyl group, C_{1-6} alkoxy group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, a group represented by the formula $-C(O)NR^{7a}R^{7b}$ (wherein R^{7a} and R^{7b} are identical to or different from each other and denote individually a hydrogen atom, or a C_{1-6} alkyl group), the formula $-CO_2R^{7a}$ (wherein R^{7a} has the same meaning as defined above), the formula $-S(O)_nR^{7a}$ (wherein n stands for an integer of 0 to 2 and

R^{7a} has the same meaning as defined above), the formula $-S(O)_2NR^{7a}R^{7b}$ (wherein R^{7a} and R^{7b} have the same meaning as defined above), a group of the formula



(wherein X^2 denotes a single bond, carbonyl group, or a group of the formula $-S(O)_2-$;

R^{5b} and R^{6b} are identical to or different from each other, and denote a hydrogen atom, a substituted or unsubstituted C_{1-6} alkyl group, or a substituted or unsubstituted C_{6-14} aryl group), or a group of the formula

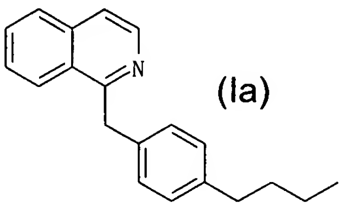
$-Z^1-Z^2$

(wherein Z^1 denotes a single bond, oxygen atom, vinylene group, or ethynylene group;

Z^2 denotes a single bond, or a C_{1-6} alkyl group substituted or unsubstituted with 0 to 4 substituents). R^{3a} and R^{4a} may together stand for a methylenedioxy group or 1,2-ethylenedioxy group, alternatively, R^{3a} and R^{4a} may together stand for the formation of a condensed ring selected from a group consisting of a substituted or unsubstituted benzene ring, substituted or unsubstituted pyridine ring, substituted or unsubstituted pyrrole ring, substituted or unsubstituted thiophene ring, substituted or unsubstituted furan ring, substituted or unsubstituted pyridazine ring, substituted or unsubstituted pyrimidine ring, substituted or unsubstituted pyrazine ring, substituted or unsubstituted imidazole ring, substituted or unsubstituted oxazole ring, substituted or unsubstituted thiazole ring, substituted or unsubstituted pyrazole ring, substituted or unsubstituted isoxazole ring, substituted or unsubstituted isothiazole ring, substituted or unsubstituted

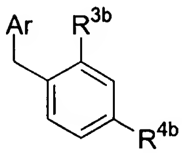
cyclohexane ring, and substituted or unsubstituted cyclopentane ring, except in cases where both R^{1a} and R^{2a} stand for hydrogen atoms.]

16. The aforementioned antifungal agent of 13, comprising as the active ingredient compound (Ia) of the formula:



(Ia)

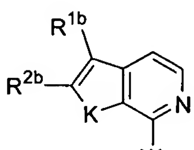
17. A compound represented by the general formula (II), a salt or a hydrate thereof, wherein in formula (II),



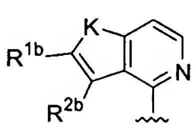
(II)

[Ar stands for a substituent selected from a group consisting of the formulae (IIIa) to (IIIf):

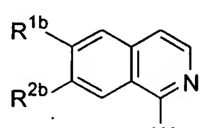
(IIIa)



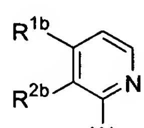
(IIIb)



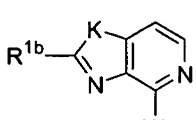
(IIIc)



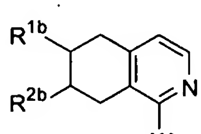
(IIId)



(IIIe)

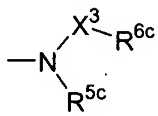


(IIIf)



(wherein K denotes a sulfur atom, oxygen atom, or a group represented by the formula -NH-;

R^{1b} and R^{2b} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro group, cyano group, trifluoromethyl group, trifluoromethoxy group, a group represented by the formula



(wherein X^3 denotes a single bond, carbonyl group, or a group represented by the formula $-S(O)_2-$;

R^{5c} and R^{6c} are identical to or different from each other and denote a hydrogen atom, or a substituted or unsubstituted C_{1-6} alkyl group), or a group represented by the formula $-X^4-R^{8a}$ (wherein X^4 denotes a single bond, oxygen atom, or sulfur atom; R^{8a} denotes a C_{1-6} alkyl group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, C_{3-8} cycloalkyl group, or C_{3-8} cycloalkenyl group). Alternatively, R^{1b} and R^{2b} may together form a methylenedioxy group, or a 1,2-ethylenedioxy group.);

R^{3b} and R^{4b} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro group, cyano group, carboxyl group, formyl group, hydroxyimino group, trifluoromethyl group, trifluoromethoxy group, C_{1-6} alkyl group, C_{1-6} alkoxy group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, or a group represented by the formula

$$-Z^{1b}-Z^{2b}$$

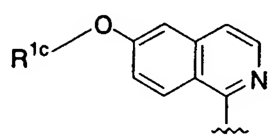
(wherein Z^{1b} denotes a single bond, vinylene group, or ethynylene group;

Z^{2b} denotes a single bond, or a C_{1-6} alkyl group that is substituted or unsubstituted with 0 to 4 substituents);

except in cases where (1) Ar stands for the aforementioned formula (IIId) wherein R^{1b} and R^{2b} are both hydrogen atoms, (2) at least

one of R^{3b} or R^{4b} denotes a hydrogen atom and the other is a hydrogen atom, methoxy group, hydroxyl group, methyl group, benzyloxy group, or a halogen atom, and Ar stands for the aforementioned formula (IIIc) wherein R^{1b} and R^{2b} both denote hydrogen atoms or methoxy groups, (3) at least one of R^{3b} or R^{4b} denotes a hydrogen atom and the other is a hydrogen atom, hydroxyl group, methoxy group, or benzyloxy group, and Ar stands for the aforementioned formula (IIIc) wherein R^{1b} and R^{2b} both denote hydroxyl groups or benzyloxy groups, or (4) Ar stands for the aforementioned formula (IIId) wherein R^{1b} is a hydrogen atom and R^{2b} is a formyl group, hydroxymethyl group, or methoxycarbonyl group.]

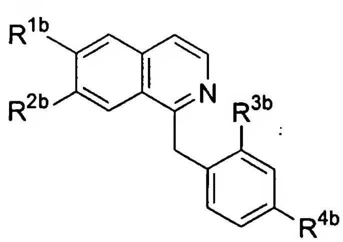
18. The compound of 17, or a salt or hydrate thereof, wherein Ar stands for the formula:



(IIIg)

(wherein R^{1c} denotes a hydrogen atom, a substituted or unsubstituted C_{1-6} alkyl group, or a benzyl group), and excluding the case when R^{3b} denotes a hydrogen atom.

19. A compound represented by the general formula (IIIc2), or a salt or hydrate thereof, wherein in formula (IIIc2),



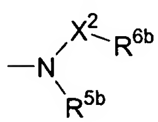
(IIIc2)

[R^{1b} and R^{2b} have the same meaning as defined above, except in cases wherein (1) R^{1b} denotes a group represented by the formula R^{1c}-O- (wherein R^{1c} has the same meaning as defined above), R^{2b} is a hydrogen atom, and R^{3b} denotes a hydrogen atom, (2) at least one

of R^{3b} or R^{4b} denotes a hydrogen atom, and the other is a hydrogen atom, methoxy group, hydroxyl group, methyl group, benzyloxy group, or a halogen atom, and R^{1b} and R^{2b} both denote hydrogen atoms or methoxy groups, or (3) at least one of R^{3b} or R^{4b} denotes a hydrogen atom, and the other is a hydrogen atom, hydroxyl group, methoxy group, or benzyloxy group, and R^{1b} and R^{2b} both denote hydroxyl groups or benzyloxy groups]

20. The antifungal agent of 17, having an antifungal action.

21. The antifungal agent of 15, wherein at least one of R^{3a} and R^{4a} denotes a group represented by the formula $-C(O)NR^{7a}R^{7b}$ (wherein R^{7a} and R^{7b} have the same meaning as defined above), the formula $-CO_2R^{7a}$ (wherein R^{7a} has the same meaning as defined above), the formula $-S(O)_nR^{7a}$ (wherein n denotes an integer of 0 to 2 and R^{7a} has the same meaning as defined above.), the formula $-S(O)_2NR^{7a}R^{7b}$ (wherein R^{7a} and R^{7b} have the same meaning as defined above), the formula



(wherein X^2, R^{5b}, and R^{6b} have the same meaning as defined above), or a C_{1-6} alkoxy group substituted or unsubstituted with 0 to 4 substituents, or R^{3a} and R^{4a} together denote a methylenedioxy group, or a 1,2-ethylenedioxy group.

22. The aforementioned antifungal agent of 15, wherein the compound having an antifungal action is (1) 1-benzylisoquinoline, (2) 1-(4-bromobenzyl)isoquinoline, (3) 1-(4-chlorobenzyl)isoquinoline, (4) 1-(4-fluorobenzyl)isoquinoline, (5) 1-(4-iodobenzyl)isoquinoline, (6) 1-(3-methylbenzyl)isoquinoline, (7) 1-(4-methylbenzyl)isoquinoline, (8) 1-(3,4-dimethylbenzyl)isoquinoline, (9) 1-(3-methoxybenzyl)isoquinoline, (10)

1-(4-methoxybenzyl)isoquinoline, (11) 1-(3,4-methylenedioxybenzyl)isoquinoline, (12) 1-(4-benzyloxybenzyl)isoquinoline, (13) 1-(4-cyanobenzyl)isoquinoline, (14) 1-(4-nitrobenzyl)isoquinoline, (15) 1-(4-aminobenzyl)isoquinoline, (16) 1-(4-methoxybenzyl)-6,7-dichloro-isoquinoline, (17) 1-(4-methoxy-2-nitro-benzyl)-isoquinoline, (18) 1-(4-methoxybenzyl)-6,7-methylenedioxy-isoquinoline, (19) 1-(2-amino-4-methoxy-benzyl)isoquinoline, (20) 1-(4-methoxybenzyl)-7-hydroxy-6-methoxy-isoquinoline, (21) 1-(4-benzyloxybenzyl)-6,7-dimethoxy-isoquinoline, (22) 1-(4-methoxybenzyl)6,7-dimethoxy-isoquinoline, (23) 1(4-methoxy-2-nitro-benzyl)-isoquinoline, (24) 3-[4-(1-isoquinolylmethyl)phenoxy]propylcyanide, (25) 1-[4-(2,2,3,3-tetrafluoropropoxy)benzyl]isoquinoline, (26) 1-[4-(2-piperidinoethoxy)benzyl]isoquinoline, (27) 4-(1-isoquinolylmethyl)phenyl(2-morpholinoethyl)ether, (28) 1-[4-(2-methoxyethoxy)benzyl]isoquinoline, (29) *N*-{2-[4-(1-isoquinolylmethyl)phenoxy]ethyl}-*N*,*N*-dimethylamine, (30) 1-[4-(phenethyloxy)benzyl]isoquinoline, (31) 1-{4-[(2-methylallyl)oxy]benzyl}isoquinoline, (32) 1-(4-isobutoxybenzyl)isoquinoline, (33) 1-[4-(2-phenoxyethoxy)benzyl]isoquinoline, (34) methyl 2-[4-(1-isoquinolylmethyl)phenoxy]acetate, (35) 2-[4-(1-isoquinolylmethyl)phenoxy]-1-ethanol, (36) t-butyl *N*-{2-[4-(1-isoquinolylmethyl)phenoxy]ethyl}carbamate, (37) 1-{4-[3-(tetrahydro-2H-2-pyranyloxy)propoxy]benzyl}isoquinoline, (38) 2-[4-(1-isoquinolylmethyl)phenoxy]-1-ethaneamine, (39) 1-[4-(3-piperidinopropoxy)benzyl]isoquinoline, (40) 3-[4-(1-isoquinolylmethyl)phenoxy]-1-propanol, (41) 1-[4-(2-ethylbutoxy)benzyl]isoquinoline, (42) 4-[4-(1-isoquinolylmethyl)phenoxy]butanoic acid, (43) 1-(4-{3-[(4-benzylpiperazino)sulfonyl]propoxy}benzyl)isoquinoline,

(44)
1-(4-{3-[4-(4-chlorophenyl)piperazino]propoxy}benzyl)isoquinoline,
(45) 4-(1-isoquinolylmethyl)aniline, (46)
N-[4-(1-isoquinolylmethyl)phenyl]butaneamide, (47)
N-[4-(1-isoquinolylmethyl)phenyl]propaneamide, (48)
N-[4-(1-isoquinolylmethyl)phenyl]-1-ethanesulfonamide, (49)
N-[4-(1-isoquinolylmethyl)phenyl]-*N*-methyl-ethanesulfonamide, (50)
N-[4-(1-isoquinolylmethyl)phenyl]-*N*-methylamine, (51)
N-[4-(1-isoquinolylmethyl)phenyl]-*N*-propylamine, or (52)
N-[4-(1-isoquinolylmethyl)phenyl]-*N*-methyl-*N*-propylamine.

23. A method for treating a mycotic infection comprising administering a therapeutically effective dose of any one of the antifungal agents of 13 to 22 to a mammal.

The present invention will be described in detail below by explaining the meaning of the terms, symbols, and such mentioned in the present description.

In the present description, the structural formula of the compounds may represent a certain isomer for convenience, however, the present invention includes all geometrical isomers, optical isomers based on asymmetric carbon, stereoisomers, and tautomers that structurally arise from compounds, and mixtures of isomers, and it is not to be construed as being limited to the representation in the formula made for convenience, and may be any one or a mixture of isomers. Therefore, an optically active substance and a racemic substance having an asymmetric carbon atom in the molecule may exist, but in this invention there are no particular limitations and any one of them are included. Furthermore, crystal polymorphism may exist, but similarly there are no limitations, and the crystal form may be any one form or may be a mixture, and may be either an anhydride or a hydrate.

Furthermore, the compounds of the present invention include compounds exhibiting antifungal action after being metabolized, such

as after being oxidized, reduced, hydrolyzed, or conjugated *in vivo*. Furthermore, the present invention includes compounds that produce the compounds of this invention after being metabolized, such as after being oxidized, reduced, and hydrolyzed *in vivo*.

The "C_{1-6} alkyl group" in the present description means a straight chain or branched chain alkyl group, wherein the number of carbon ranges from 1 to 6, and specific examples include a methyl group, ethyl group, *n*-propyl group, *i*-propyl group, *n*-butyl group, *i*-butyl group, *tert*-butyl group, *n*-pentyl group, *i*-pentyl group, neopentyl group, *n*-hexyl group, 1-methylpropyl group, 1,2-dimethylpropyl group, 2-ethylpropyl group, 1-methyl-2-ethylpropyl group, 1-ethyl-2-methylpropyl group, 1,1,2-trimethylpropyl group, 1-methylbutyl group, 2-methylbutyl group, 1,1-dimethylbutyl group, 2,2-dimethylbutyl group, 2-ethylbutyl group, 1,3-dimethylbutyl group, 2-methylpentyl group, 3-methylpentyl group, and so on.

The "C_{2-6} alkenyl group" in the present description means a straight chain or branched chain alkenyl group, wherein the number of carbon ranges from 2 to 6, and specific examples include a vinyl group, allyl group, 1-propenyl group, isopropenyl group, 1-butene-1-yl group, 1-butene-2-yl group, 1-butene-3-yl group, 2-butene-1-yl group, 2-butene-2-yl group, and so on.

The "C_{2-6} alkynyl group" in the present description means a straight chain or branched chain alkynyl group, wherein the number of carbon ranges from 2 to 6, and specific examples include an ethynyl group, 1-propynyl group, 2-propynyl group, butynyl group, pentynyl group, hexynyl group, and so on.

The "C_{1-6} alkoxy group" in the present description means an oxy group to which "C_{1-6} alkyl group" defined above is bound, and specific examples include a methoxy group, ethoxy group, *n*-propoxy group, *i*-propoxy group, *n*-butoxy group, *i*-butoxy group, *sec*-butoxy group, *t*-butoxy group, *n*-pentyloxy group, *i*-pentyloxy group, *sec*-pentyloxy group, *t*-pentyloxy group, neopentyloxy group, 1-methylbutoxy group, 2-methylbutoxy group, 1,1-dimethylpropoxy group, 1,2-dimethylpropoxy

group, *n*-hexyloxy group, *i*-hexyloxy group, 1-methylpentyloxy group, 2-methylpentyloxy group, 3-methylpentyloxy group, 1,1-dimethylbutoxy group, 1,2-dimethylbutoxy group, 2,2-dimethylbutoxy group, 1,3-dimethylbutoxy group, 2,3-dimethylbutoxy group, 3,3-dimethylbutoxy group, 1-ethylbutoxy group, 2-ethylbutoxy group, 1,1,2-trimethylpropoxy group, 1,2,2-trimethylpropoxy group, 1-ethyl-1-methylpropoxy group, 1-ethyl-2-methylpropoxy group, and so on.

The "C_{6-14} aryl group" in the present description refers to an aromatic ring group, wherein the number of carbon ranges from 6 to 14, and specific examples include a phenyl group, 1-naphthyl group, 2-naphthyl group, *as*-indacenyl group, *s*-indacenyl group, acenaphthylenyl group, and so on.

The "halogen atom" of the present description means a fluorine atom, chlorine atom, bromine atom, and iodine atom.

"Substituted or unsubstituted" in the present description means "the substitutable site may have an arbitrary combination of one or more substituents" and specifically the substituents are, for example, a hydrogen atom, halogen, nitro group, cyano group, hydroxyl group, mercapto group, hydroxyalkyl group, carboxyl group, C_{1-6} alkoxycarbonyl group, C_{2-7} acylamino group, C_{1-6} alkylamino group, pyridyl group, C_{1-6} alkylsulfinyl group, C_{1-6} alkylsulfonyl group, C_{1-6} alkylsulfamoyl group, C_{1-6} alkylsulfinamoyl group, C_{1-6} alkylsulfenamoyl group, tetrahydropyranyl group, C_{1-6} alkylcarbamoyl group, or the formula $-X^4-R^{8a}$ (wherein X^4 denotes a single bond, oxygen atom, or sulfur atom; R^{8a} denotes a C_{1-6} alkyl group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, C_{6-14} aryl group, C_{3-8} cycloalkyl group, or C_{3-8} cycloalkenyl group), and so on.

"May be substituted with 0 to 4 substituents" has the same meaning as "the substitutable site may have an arbitrary combination of 1 to 4 substituents" and the substituents have the same meaning as defined above.

"Salt" in the present invention refers to a pharmaceutically

acceptable salt, and there are no particular limitations as long as the salt has formed an addition salt with a compound of this invention, and a preferred example is a haloid acid salt such as hydrofluoride, hydrochloride, hydrobromide, and hydroiodide; an inorganic acid salt such as a sulfate, nitrate, perchlorate, phosphate, carbonate, and bicarbonate; an organic carboxylate such as an acetate, oxalate, maleate, tartrate, and fumarate; an organic sulfonate such as a methanesulfonate, trifluoromethanesulfonate, ethanesulfonate, benzenesulfonate, toluenesulfonate, and camphorsulfonate; an amino acid salt such as an aspartate, and glutamate; salts with an amine such as a trimethylamin, triethylamine, procaine, pyridine, and phenethylbenzylamine; alkali metal salts such as sodium, and potassium; alkaline earth metal salts such as magnesium and calcium; and so on.

Herein below, the following will be disclosed: 1. A method for obtaining DNAs encoding proteins participating in cell wall synthesis, 2. a method for examining whether or not a test sample influences the process that transports GPI-anchored proteins to the cell wall, and 3. a method for obtaining the aforementioned compound (Ia) of the present invention.

1. A method for obtaining DNAs encoding proteins participating in fungal cell wall synthesis

Hereinafter, (1) a method for obtaining a DNA encoding a protein for acquiring resistance to the aforementioned compound (Ia) by overexpression in fungi; (2) a method for obtaining a DNA that hybridizes under stringent conditions with the DNA of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 5; (3) a method for obtaining a DNA that encodes a protein that participates in fungal cell wall synthesis, based on a homology search; and (4) a method for obtaining a fungus that overexpressed or lacked the protein for acquiring resistance to the aforementioned compound (Ia), will be described.

(1). A method for obtaining a DNA encoding a protein for acquiring resistance to the aforementioned compound (Ia) by overexpression of the

DNA in a fungus

Herein, “fungus” means a fungus belonging to Division Zygomycota, Ascomycota, Basidiomycota, and Deuteromycota. Preferable is a pathogenic fungus, *Mucor*, *Saccharomyces*, *Candida*, *Cryptococcus*, *Trichosporon*, *Malassezia*, *Aspergillus*, *Trichophyton*, *Microsporum*, *Sporothrix*, *Blastmyces*, *Coccidioides*, *Paracoccidioides*, *Penicillinium*, or *Fusarium*, and more preferable is *C. albicans*, *C. glabrata*, *C. neoformans*, or *A. fumigatus*. *S. cerevisiae* and *S. pombe*, for which genetic analyses are easy, are also preferred strains.

A plasmid library of a fungal gene is introduced into a fungus. The plasmid library of *S. cerevisiae* and *S. pombe* can be obtained from ATCC (Information for ATCC Number: 37323), and the plasmid library of *C. albicans* can be produced by the method according to Navaro-Garcia, F. et al, Mol. Cell. Biol., 15: 2197-2206, 1995. The obtained plasmid library is introduced to the fungi by the method according to Gietz, D. et al, Nucl. Acids Res. 20: 1425, 1992. Alternatively, a kit such as YEASTMAKER™ Yeast Transformation System (Clontech) may be used.

The Fungus to which the plasmid library is introduced is cultured in the presence of the aforementioned compound (Ia). Specifically, an agar medium containing the aforementioned compound (Ia) at a concentration of 1.56 to 25 μg/ml, preferably 1.56 to 6.25 μg/ml, and more preferably 3.125 μg/ml is inoculated with the fungus into which a plasmid library has been introduced, is cultured for an appropriate length of time, at 30°C to 42°C for 2 to 5 days, or preferably at 37°C for 3 days. The colony formed upon proliferation is further cultured in a medium containing the aforementioned compound (Ia), and the plasmid is purified from the proliferated fungal cells. Purification of the plasmid can be performed by the method according to METHODS IN ENZYMOLOGY, Vol. 194: 169-182 (1991), for example.

Preferably, the nucleotide sequence of the obtained plasmid is determined directly, but if necessary, cloning into an appropriate vector, for example pBluescript II, and pUC19 suitable for nucleotide sequence determination, is done to determine the nucleotide sequence.

A nucleotide sequence can be determined for example by the method accompanying the ABI377 System (PE applied Biosystems) manual.

In the Examples of the present invention, all 27 of the independently obtained colonies of *S. cerevisiae*, and 28 colonies out of 30 colonies of *C. albicans* contained the DNAs of this invention. Only one gene that confers resistance to the aforementioned compound (Ia) exists in these fungi and this can be obtained by the abovementioned method.

(2). A method for obtaining a DNA that hybridizes under stringent conditions to the DNA of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 5

An example of a method for obtaining a DNA encoding a protein participating in fungal cell wall synthesis according to the present invention comprises designing a primer from the information of the nucleotide sequence of SEQ ID NO: 1 using the genomic DNA of *S. cerevisiae* as a template, or designing a primer from the information of the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 5 using the genomic DNA of *C. albicans* as the template, then performing PCR, and cloning the amplified DNA into an appropriate vector, such as pBlueScript. The primer is designed as necessary according to the region to be amplified, and the length is preferably 15 bp or more, more preferably 20 bp or more, and in some cases sequences necessary for subsequent DNA construction, such as restriction enzyme sites, may be added. The conditions for PCR can be determined appropriately according to factors such as the length of primer, the length of the region to be amplified, and the amount of template DNA to be used. For example, a DNA encoding a protein participating in cell wall synthesis in a fungus can be obtained using 200 ng of the genomic DNA of *C. albicans* as a template, and SEQ ID NO: 21 and SEQ ID NO: 22 as primers under conditions of 94°C for 4 minutes → (94°C for 30 seconds → 68°C for 5 minutes) x 35 cycles → 72°C for 4 minutes.

The DNA obtained by PCR may be used as a probe for obtaining other types of fungal DNA showing homology to the DNA encoding the protein participating in cell wall synthesis. Specifically, for example, to

obtain a homologous gene of *C. albicans* encoding the protein participating in *S. cerevisiae* cell wall synthesis, DNA that hybridizes under stringent conditions can be cloned from the genomic library or cDNA library of *C. albicans*, using the genomic DNA of *S. cerevisiae* as a template, and using DNA that is obtained by PCR as a probe. Herein, stringent conditions refer to hybridization in 4x SSC at 65°C, then washing in 0.1x SSC at 65°C for 1 hour, for example. Furthermore, in another the stringent conditions are 4x SSC at 42°C in 50% formamide. Alternatively, conditions such as hybridization in the PerfectHyb™ (TOYOBO) solution at 65°C for 2.5 hours, then washing in 1). 2x SSC, 0.05% SDS solution at 25°C for 5 minutes, 2). 2x SSC, 0.05% SDS solution at 25°C for 15 minutes, and 3). 0.1x SSC, 0.1% SDS solution at 50°C for 20 minutes, are also allowed.

The Examples of this invention demonstrate from Southern Blot analysis that there is only one gene in *C. albicans* that hybridizes with the DNA of SEQ ID NO: 1, and shows the cloning of this gene. From the above-mentioned method, DNA that hybridizes with SEQ ID NO: 1 or SEQ ID NO: 3 can be obtained.

(3). A method for obtaining a DNA that encodes a protein that participates in fungal cell wall synthesis, based on a homology search

The present invention revealed the GWT1 homologues of *S. cerevisiae*, *C. albicans*, *S. pombe*, *A. fumigatus*, and *C. neoformans*. The region conserved among these genes is considered to be important for GWT1 gene products to exhibit their function, and may very well be conserved in other fungi.

Therefore, a DNA encoding a protein participating in fungal cell wall synthesis can be obtained by either carrying out hybridization upon constructing a probe based on the amino acid sequence of the conserved region, or by performing PCR by designing primers based on the sequence. The PCR primer may be of any sequence as long as it is designed to encode the conserved region, but is preferably SEQ ID NOS: 29 and 31 or preferably SEQ ID NOS: 29 and 30.

Furthermore, as another method, a DNA encoding a protein participating in fungal cell wall synthesis can be obtained by carrying out PCR with cDNA or genomic DNA upon finding a nucleotide sequence showing homology to GWT1 from gene fragments registered in databases, and then designing primers based on that nucleotide sequence.

Examples of PCR methods for obtaining a full-length gene based on the obtained sequence are techniques such as 3'-RACE, 5'-RACE, and inverse PCR, and it is also possible to select by hybridization a clone containing neighboring sequences. A full-length gene can be obtained by combining these techniques.

(4). a method for obtaining a fungus that overexpresses or lacks a protein for acquiring resistance to the aforementioned compound (Ia)

A Fungus, preferably *S. cerevisiae*, that overexpresses a protein for acquiring resistance to the aforementioned compound (Ia) of this invention can be obtained by the method of inserting an expression vector expressing the protein into a particular position on the fungal chromosome, for example an expression vector in which the DNA of SEQ ID NO: 1 is connected downstream of a promoter, which can forcibly express the protein in fungi, preferably the promoter of budding yeast enolase gene (ENO1). The insertion method can be performed, for example, by the steps of, inserting a desired sequence into the multicloning site of pRS304 (Sikorski RS et al, Genetics. 122(1): 19-27, 1989), constructing a vector for integration, and introducing the vector into the fungus. One can refer to METHODS IN ENZYMOLOGY Vol.194: 281-301 (1991) for details.

Furthermore, an overexpressed strain of *C. albicans* can be obtained by incorporating the gene of SEQ ID NO: 3 or SEQ ID NO: 5 into an expression vector for *C. albicans*, such as pCARS1 and pRM1(Pla J et al, Yeast 12: 1677-1702, 1996), and then transforming *C. albicans* (Sanglard D et al, Antimicrobiol. Agents Chemother. 40: 2300-2305, 1996).

Fungi of this invention lacking a gene for acquiring resistance

against the aforementioned compound (Ia), preferably *S. cerevisiae*, can be obtained by the following methods, but is not to be construed as being limited thereto.

PCR amplification is carried out using a marker gene, preferably his5 gene of *S. pombe*, as a template, and using primers that are designed so that PCR products that contain the gene to be deleted (30 bp or more, or preferably 40 bp or more). In the case of *S. cerevisiae*, the genetic sequence of SEQ ID NO: 1, positioned on both ends can be obtained. The PCR products can be purified and introduced into fungi, then cultured in a selection medium corresponding to the marker gene, for example, his^- for his5, to obtain the deletion strain.

Furthermore, the deletion strain of *C. albicans* is obtained by the usual method using a hisG-URA3-hisG cassette (Fonzi WA et al, Genetics 134: 717-728,1993) based on the nucleotide sequence information of SEQ ID NO: 3 or SEQ ID NO: 5.

2. A method for examining whether or not the test sample influences the process that transports GPI-anchored proteins to the cell wall

Whether or not the test sample inhibits the process that transports GPI-anchored proteins to the cell wall, or whether or not the test sample inhibits the expression of the GPI-anchored protein in the fungal surface can be examined by (1) a method using a reporter enzyme, (2) a method using an antibody that reacts with the surface glycoprotein of the fungal cell wall, (3) a method for examining the adhesion ability towards animal cells, and (4) a method for observing fungi using an optical microscope or an electron microscope.

By using the methods of (1) to (4) described below, preferably the methods of (1) to (4) in combination, the test sample is judged to inhibit the process that transports GPI-anchored proteins to the cell wall, or the expression of the GPI-anchored proteins at the fungal surface. Furthermore, it is judged that the test sample influences the process that transports GPI-anchored proteins to the cell wall when the degree of inhibition diminishes or the inhibition is no longer seen when

the protein encoded by the DNA of the present invention is overexpressed in fungi.

Hereinafter, the methods of (1) to (4) will be described.

(1). A method using a reporter enzyme

The process that transports GPI-anchored proteins to the cell wall can be quantified by a tracer experiment such as labeling a GPI-anchored protein with a radioactive isotope, then upon fractionation of the fungal cell wall fraction, immunoprecipitating with an antibody against a GPI-anchored protein. Alternatively, the quantification can be more readily done by expressing the C-terminal sequence considered to function as a transport signal, which is commonly observed among GPI-anchored proteins, as a fusion protein with an easily measurable enzyme (reporter enzyme), fractionating the fungal cell wall fraction, and then using a reporter system that measures the enzyme activity of each fraction (Van Berkel MAA et al, FEBS Letters, 349: 135-138, 1994). Hereinafter, a method using the reporter enzyme will be explained, but the present invention is not to be construed as being limited thereto.

First, the reporter gene is constructed and is introduced into a fungus. The reporter gene is constructed by linking a promoter sequence that functions in fungi, followed by DNAs that respectively encode a signal sequence, a reporter enzyme, and a GPI-anchored protein C-terminal sequence so that the reading frames match. Examples of the promoter sequences are those of promoters such as GAL10, and ENO1. Examples of signal sequences are those of α-factor, invertase, lysozyme, and such. Examples of reporter enzymes are β-lactamase, lysozyme, alkaline phosphatase, β-galactosidase, and such. Green Fluorescence Protein (GFP), which can be detected easily, can be used, even though it does not have enzyme activity. Examples of GPI-anchored protein C-terminal sequences are α-agglutinin C-terminal sequence, CWP2 C-terminal sequence, and such. Furthermore, it is preferable to insert an appropriate selection marker such as LEU2, and URA3 into the vector containing the constructed reporter gene.

The constructed reporter gene is inserted into a fungus by an

appropriate method, such as the lithium acetate method (Gietz D et al, Nucl. Acids Res. 20: 1425, 1992), and cultured, if necessary by a method suitable for the selection marker, such as Leu^- medium for LEU2, and Ura^- medium for URA3, and then fungi into which the DNA has been introduced are selected.

Whether or not a test sample influences the process that transports GPI-anchored proteins to the cell wall is examined by the following method.

The reporter gene-introduced fungi are cultured under appropriate conditions, for example at 30°C for 48 hours, in the presence of a test sample. After culturing, the culture supernatant is centrifuged, and the reporter enzyme activity of the culture supernatant fraction is measured. The remaining cell fraction is washed, then the cell wall components are separated by an appropriate method, such as degrading the cell wall glucan with glucanase, and then measuring the reporter enzyme activity of the cell wall fraction and the cytoplasmic fraction. The assay can be simply carried out by determining the amount of reporter enzyme in the cell fraction by centrifuging, then without washing the cells, determining the amount of reporter enzyme derived from the culture supernatant fraction that remains in the cell fraction by proportional calculation, and subtracting this from the amount of reporter enzyme of the cell fraction.

If an activity to increase the reporter enzyme activity within the culture supernatant fraction (activity per cell), or an activity to decrease the reporter enzyme activity in the cell wall fraction (activity per cell) is confirmed in the test sample, the test sample is judged to have influenced the process that transports GPI-anchored proteins to the cell wall.

(2). A method using an antibody that reacts with the surface glycoprotein of a fungal cell wall

Whether or not the test sample influences the expression of the GPI-anchored protein at the fungal surface layer can be detected by quantifying a GPI-anchored protein in the fungal cell wall using an

antibody that reacts with the protein.

For example, as the antibody, the antigenic determinant is predicted from the amino acid sequence of a GPI-anchored protein , for example, α-agglutinin, Cwp2p, and Als1p (Chen MH et al, J. Biol. Chem., 270:26168-26177, 1995; Van Der Vaat JM et al, J. Bacteriol., 177:3104-3110,1995; Hoyer LL et al, Mol. Microbiol., 15:39-54, 1995), the peptide of that region is synthesized, this is bound to an antigenic substance, such as a carrier protein, and then polyclonal antibodies can be obtained by immunizing a rabbit and such, or a monoclonal antibody can be obtained by immunizing a mouse and such. Furthermore, a house rabbit polyclonal antibody against the Als1p peptide is preferable.

In an alternative method, a monoclonal antibody against a GPI-anchored protein may be obtained by immunizing a mouse and such with a fungus, preferably a fungus overexpressing the GPI-anchored protein, such as α-agglutinin, Cwp2p, and Als1p, and in some cases, by immunizing with the partially purified GPI-anchored protein , and selecting the clone yielded as a result of the fusion by ELISA, Western blot analysis, and such.

Whether or not the test sample influences the process that transports GPI-anchored proteins to the cell wall, and diminishes the amount of the protein derived from the GPI-anchored protein in the cell wall can be examined by the following method.

A fungus is cultured in the presence of a test sample under appropriate conditions, such as 30°C, for 48 hours. The cultured fungus is collected by centrifugation and the cells are disrupted, preferably using glass beads. The washed, disrupted cells are preferably subjected to centrifugal extraction with SDS, then the precipitate is washed. After the extraction, the disrupted cells are treated with an enzyme that degrades glucan, preferably glucanase, and the centrifuged supernatant thereof is the GPI-anchored protein sample.

The anti-Als1p peptide antibody is coated onto a 96-well plate by incubating at 4°C overnight. After washing with a washing solution, preferably PBS containing 0.05% Tween 20 (PBST), blocking is carried

out with a reagent that blocks the non-specific adsorption sites of the 96-well plate, preferably a protein such as BSA, and gelatin, more preferably BlockAce. After washing again with a washing solution, preferably PBST, in some cases, after adding an appropriately diluted GPI-anchored protein sample, the reaction is carried out for an appropriate length of time, such as 2 hours at room temperature. After washing with a washing solution, preferably with PBST, an antibody against the enzyme-labeled *C. albicans*, preferably HRP-labeled anti-*Candida* antibody, is reacted for an appropriate length of time, such as 2 hours at room temperature. The method for labeling may be enzyme labeling or radioactive isotope labeling. After washing with a washing solution, preferably PBST, the amount of Als1p in the GPI-anchored protein sample is calculated by a method appropriate for the type of label, i.e. for an enzyme label, adding a substrate solution, and then upon stopping the reaction, measuring the absorbance at 490 nm.

(3). A method for examining the adhesion ability towards animal cells

Whether or not the test sample influences expression of a GPI-anchored protein on the fungal surface can be examined by measuring the activity of the GPI-anchored protein in the fungal cell wall, preferably by measuring the adhesion ability of fungi to animal cells, and such. Besides Als1p, Hwp1p, and such participating in adhesion to animal cells, α-agglutinin participating in mating, Flo1p participating in yeast aggregation, and such are known as GPI-anchored proteins. Hereinafter, examination methods that use the adhesion ability of fungi to animal cells will be explained in detail, but this invention is not to be construed as being limited thereto.

As the fungus, a fungus having an adhesion ability towards cells is used, and preferably, the fungus is *C. albicans*. For mammalian cells, cells that adhere to the fungus are used, and preferably, are intestinal epithelial cells. The mammalian cells are cultured and are immobilized by an appropriate method such as ethanol immobilization. The test sample and the fungi, which have been incubated for an appropriate length

of time, such as 48 hours at 30°C, are inoculated, then after culturing for a certain length of time, for example 1 hour at 30°C, the culture supernatant is removed, washed with a buffer, and is superposed onto an agar media, such as Sabouraud Dextrose Agar Medium (Difco). After culturing at 30°C overnight, the number of colonies is counted, and the adhesion rate is calculated.

If activity to lower the number of colonies formed by adhesion of fungi to cells is observed in a test sample compared to that of fungi that are not treated with the compound, the test sample is judged to have influenced the process that transports GPI-anchored proteins to the cell wall.

(4). A method for observing fungi using an electron microscope or an optical microscope

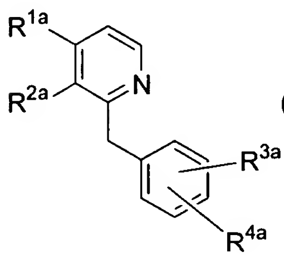
Whether or not a test sample influences the expression of the GPI-anchored proteins in the fungal surface can be examined by observing the structure of the fungal cell wall using an electron microscope.

In the presence of a test sample, a fungus such as *C. albicans* is cultured for a certain length of time, for example, 48 hours at 30°C, and the ultrafine morphological structure is observed with a transmission electron microscope. Herein, observation with a transmission electron microscope can be carried out, for example by the method according to the Electron Microscope Chart Manual (Medical Publishing Center). The flocculent fibrous structure of the outermost layer of the fungal cell that has a high electron density and is observable by transmission electron microscope image, is considered to be a surface glycoprotein layer having GPI-anchored proteins as its constituents, and is not influenced by other existing antifungal agents. When this flocculent fibrous structure of the outermost layer of a fungal cell, which has a high electron density, disappears leaving a slight layer with a high electron density, compared to that in the untreated cells, the test sample is judged to have influenced the process that transports GPI-anchored proteins to the cell wall.

When images, in which fungal cells are largely swollen and budding

(division) is inhibited, are observed under a transmission electron microscope in addition to an optical microscope, the test sample is judged to have an influence on the cell wall.

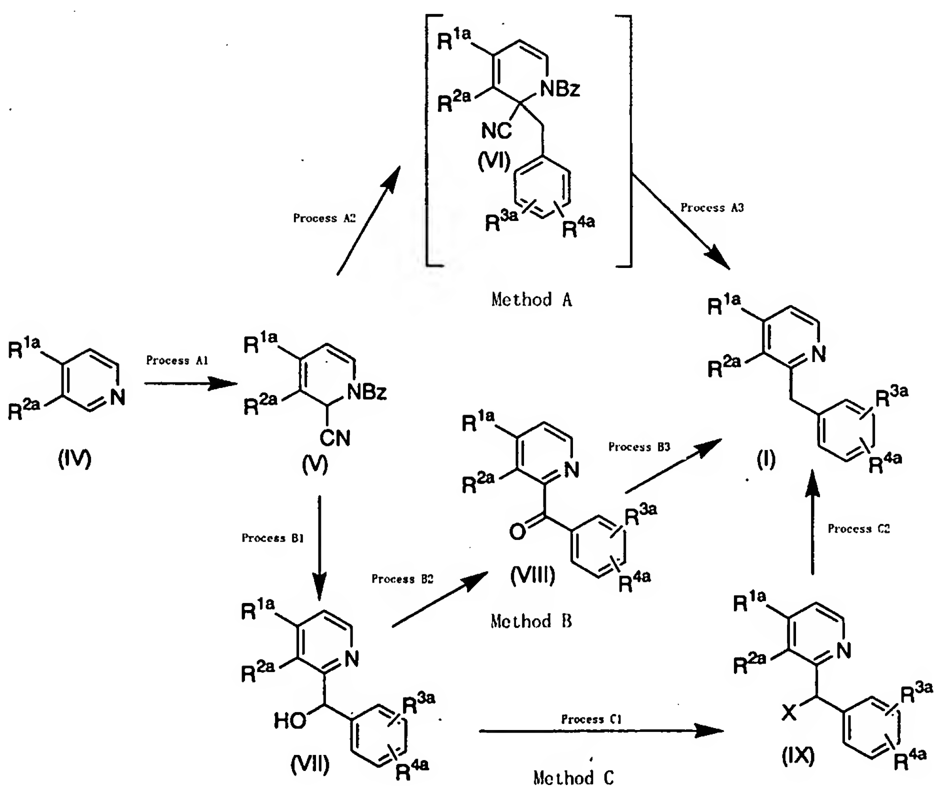
The compounds of the present invention represented by the formula (I)



(I)

(wherein the symbols have the same meaning as defined above) can be synthesized by utilizing conventional organic chemical reactions and such that have been known to date. For example, it can be synthesized by the following methods.

<u>Production method (1)</u>



In the above formulae, X is a leaving group such as a halogen group and acyl group. R^{3c} has the same meaning as R^{3a}. Other symbols in the formulae have the same meaning as defined above.

Process A1

A reaction for producing the Reissert compound (V). The compound can be produced based on the reaction conditions according to the literature, such as Org. Synth., VI, 115(1988); Heterocycles, 36(11), 2489(1993); J. Chem. Soc. (C), 666(1969); or J. Heterocycl. Chem., 29(5), 1165(1992). Specifically, the reagents used are, for example, a combination of benzoyl chloride and potassium cyanide.

Process A2

A process for alkylation. The compound (VI) can be produced by reacting the compound (V) with a substituted benzyl halide derivative, a substituted benzylmethanesulfonate derivative, or such in the presence of a base. Specific examples of the base include sodium hydride, sodium hydroxide.

Process A3

A process for hydrolysis reaction. The compound (I) can be produced by hydrolysis of the compound (VI) in the presence of a base.

Method A is a method for producing the compound (I) via Process A1, Process A2, and Process A3.

Process B1

A process for conversion of the compound (V) to the compound (VII). The compound (VII) can be produced by reacting the compound (V) with a substituted benzaldehyde in the presence of a base and a phase-transfer catalyst. Examples of the base include sodium hydroxide and potassium hydroxide. Examples of the phase-transfer catalyst include triethylbenzylammonium chloride.

Process B2

A process for oxidation of the alcohol to the ketone. The ketone derivative (VIII) can be produced by using an oxidizing agent and a condition conventionally used for the oxidation reaction of an alcohol to a ketone. Specifically, the oxidizing agent is, for example,

manganese dioxide, chromium dioxide, or benzoquinone.

Process B3

A process for reduction of the ketone to the methylene. The methylene derivative (I) can be produced by using a conventionally used combination of reducing agents for the reduction reaction of the ketone derivative (VIII) to the methylene derivative (I). Examples of the combination of the reducing agents include hydrazine hydrate and sodium hydroxide or potassium hydroxide, triethylsilane and boron trifluoride, and trifluoromethanesulfonic acid.

Method B is a method for producing the compound (I) via Process A1, Process B1, Process B2, and Process B3.

Process C1

A process for halogenation or acylation of the hydroxyl group. The compound (IX) can be produced by reacting a halogenating agent or an acylating agent with the compound (VII). Examples of the halogenating agent include thionyl chloride, concentrated hydrochloric acid, and phosphorus tribromide. Furthermore, examples of the acylating agent include acid halides such as acetyl chloride and acid anhydrides such as acetic anhydride.

Process C2

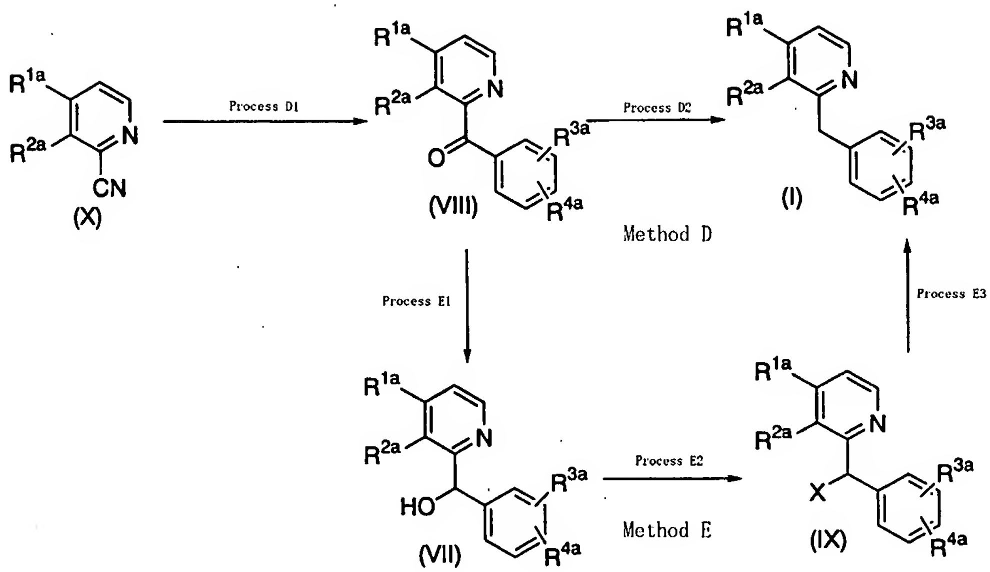
A process for reductive elimination reaction of the halogen group or the acyl group. The compound (I) can be produced by hydroelimination of the compound (IX), for example, by using a catalyst.

Examples of the catalyst include palladium-carbon.

Method C is a method for producing the compound (I) via Process A1, Process B1, Process C1, and Process C2.

Production method (2)

The compound of the present invention represented by the formula (I) can also be synthesized by the following method.



In the formula, X is a leaving group such as a halogen group and acyl group. Other symbols in the formulae have the same meaning as defined above.

Process D1

A process for a Grignard reaction and a subsequent acid hydrolysis reaction. The compound (VIII) can be produced by reacting the compound (X) with a substituted or unsubstituted phenyl Grignard reagent, followed by hydrolysis in the presence of an acid.

Process D2

The methylene derivative (I) can be produced from the ketone derivative (VIII) by conditions similar to that of Process B3.

Method D is a method for producing the compound (I) via Process D1 and Process D2.

Process E1

A process for the reduction reaction from the ketone to the alcohol. The compound (VII) can be produced from the compound (VIII) using a reducing agent and conditions conventionally used for the reduction reaction of a ketone to an alcohol. Specific examples of the reducing agent include sodium borohydride and lithium aluminum hydride.

Process E2

Under conditions similar to that of Process C1, the halogenated or acylated derivative (IX) can be produced from the alcohol derivative (VII).

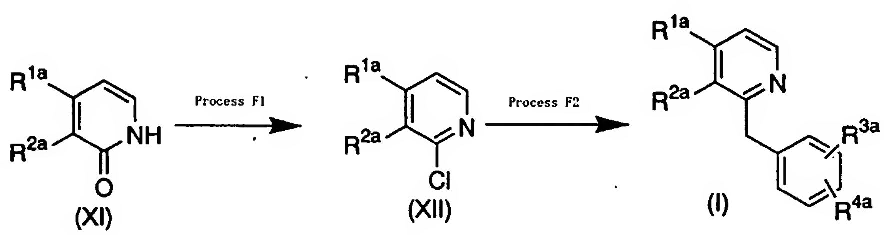
Process E3

Under conditions for reductive elimination reaction similar to that of Process C2, the compound (I) can be produced from the compound (IX).

Method E is a method for producing the compound (I) via Process D1, Process E1, Process E2, and Process E3.

Production method (3)

The compound of the present invention represented by the formula (I) can also be synthesized by the following method.



Method F

The symbols in the formulae have the same meaning as defined above.

Process F1

A process for the chlorination reaction. The compound (XII) can be produced by reacting the compound (XI) with a chlorinating agent. Examples of the chlorinating agent include phosphorus oxychloride and thionyl chloride.

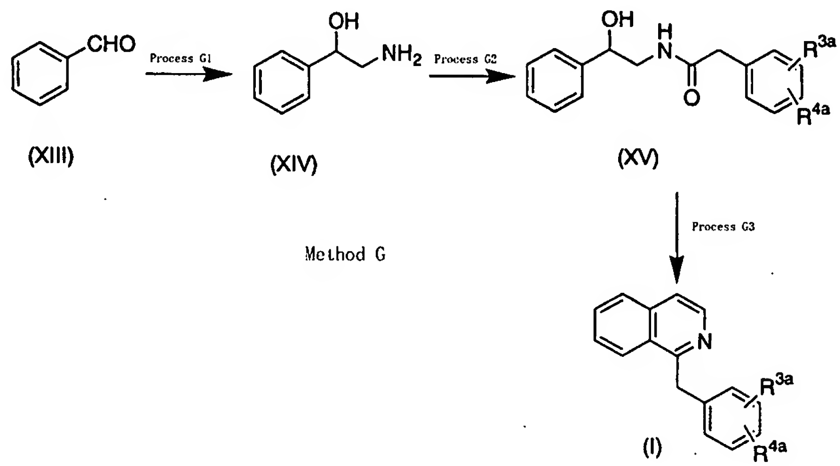
Process F2

A process for the coupling reaction with a Grignard reagent. The compound (I) can be produced by reacting the compound (XII) with a substituted or unsubstituted benzyl Grignard reagent in the presence of a catalyst, based on the reaction conditions according to the literature, such as Arch. Pharm, 314, 156(1981). Examples of the catalyst include [1,1'-bis(diphenylphosphino)ferrocene]dichloro nickel(II).

Method F is a method for producing the compound (I) via Process F1 and Process F2.

Production method (4)

The compound of the present invention of the formula (I), wherein R^{1a} and R^{2a} together form a condensed ring such as a benzene ring, pyridine ring, pyrrole ring, thiophene ring, furan ring, cyclohexane ring, or cyclopentane ring, can be synthesized by the following method.



Method G

The symbols in the formulae have the same meaning as defined above.

The production method in which the isoquinoline ring is formed is shown below as an example.

Process G1

A process for the condensation reaction and the subsequent reduction reaction. The compound (XIV) can be produced by a condensation reaction between the substituted or unsubstituted benzaldehyde derivative (XIII) and nitromethane, followed by reduction of the nitro group. Examples of the reagent used for the reduction of the nitro group include a combination of palladium-carbon and ammonium formate, and lithium aluminum hydride.

Process G2

An amide bond formation reaction. The compound (XV) can be produced by reacting the compound (XIV) and a substituted or unsubstituted phenylacetyl chloride with a coupling reagent for an amide

bond formation reaction. Examples of the coupling reagent include a combination of *N,N'*-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide, a combination of *N,N'*-dicyclohexylcarbodiimide and *N*-hydroxybenzotriazole, and 1,1'-carbonyldiimidazole.

<u>Process G3</u>

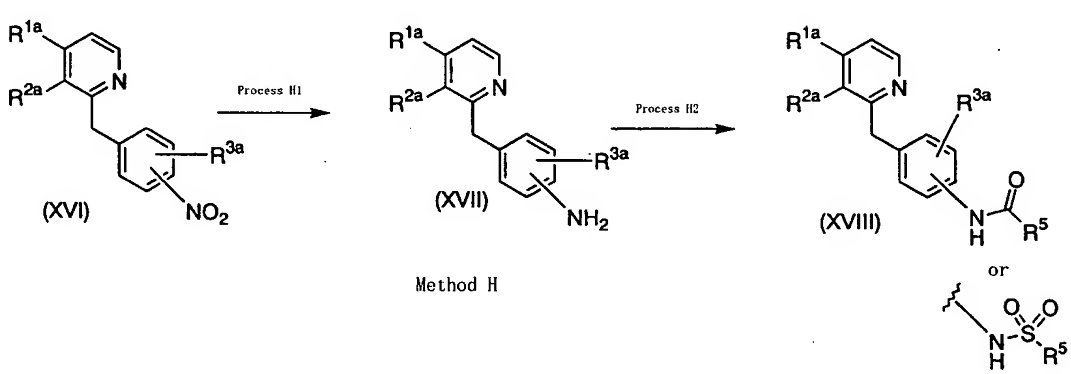
A process for the cyclization reaction. The compound (XV) can be produced based on the reaction conditions according to the literature, such as Organic Reaction, 6, 74(1951); J. Hetetocyclic Chem., 30, 1581(1993). Examples of the reagent for this reaction include phosphorus oxychloride and polyphosphoric acid.

Method G is a method for producing the compound (I) via Process G1, Process G2, and Process G3.

<u>Production method (5-1)</u>

Replacement of the substituent R^{3a} or R^{4a} of the compound (I) synthesized by the aforementioned production method

(5-1) Replacement of the substituent with an amino group, amide group, sulfonamide group, etc.



The symbols in the formulae have the same meaning as defined above.

<u>Process H1</u>

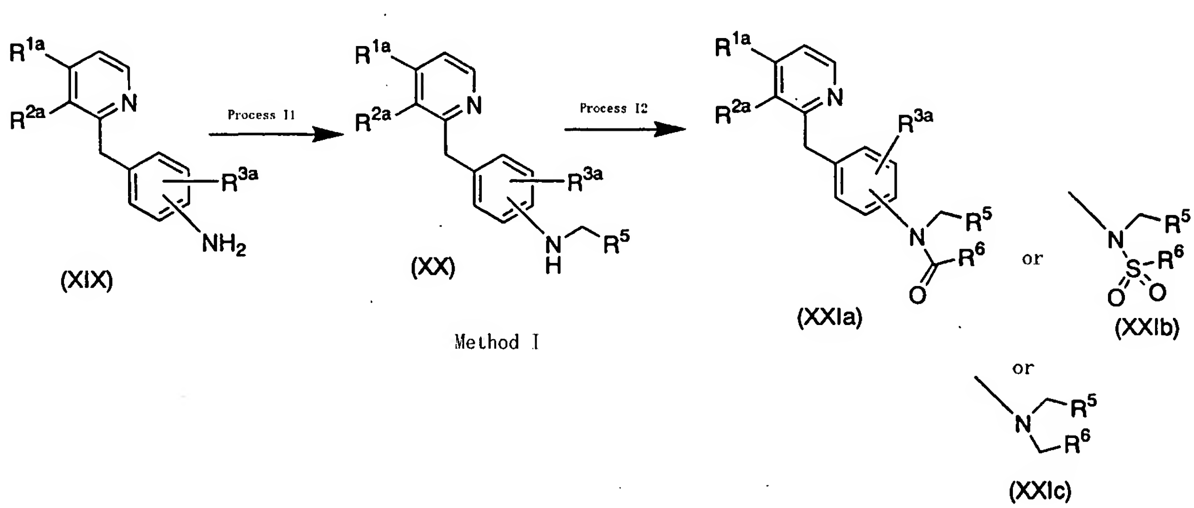
A reduction reaction of the nitro group. The compound (XVII) can be produced by reducing the compound (XVI) with a conventionally used method for reduction of a nitro group. Examples of the reduction method are catalytic hydrogenation reduction by palladium-carbon, or palladium hydroxide, and reduction by iron-ammonium chloride, iron-hydrochloric

acid, iron-acetic acid, etc.

Process H2

A process for the acylation or sulfonylation reaction. The compound (XVIII) can be produced by treating the compound (XVII) with an acid chloride or acid anhydride.

Method H is a method for producing the compound (XVIII) via Process H1 and Process H2.



Method I

The symbols in the formulae have the same meaning as defined above.

Process I1

A process for the reductive amination reaction. The compound (XX) can be produced from the compound (XIX) and a substituted or unsubstituted aldehyde based on the reaction conditions according to the literature, such as J. Am. Chem. Soc., 93, 2897(1971); Comprehensive Organic Synthese, 8, 25(1991); Tetrahedron, 40, 1783(1984); and Tetrahedron, 41, 5307(1985). Examples of the reductive amination reagent include sodium triacetoxyhydroborate, sodium cyanotrihydroborate, borane-pyridine complex, and palladium-carbon/hydrogen.

Process I2

A process for the acylation, sulfonylation, or reductive amination reaction. The compound (XXIa) or the compound (XXIb) can be produced from the compound (XX) using an acid chloride or an acid anhydride. The

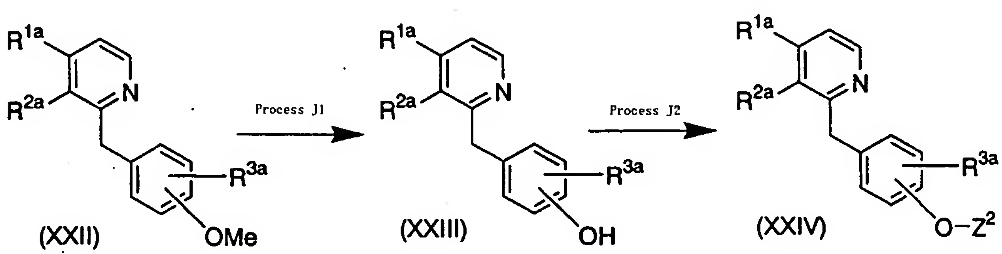
compound (XXIc) can be produced by carrying out a reductive amination reaction similarly to that of Process I1.

Method I is a method for producing the compound (XXIa), the compound (XXIb), or the compound (XXIc) via Process I1 and Process I2.

Production method (5-2)

Replacement of the substituent R^{3a} or R^{4a} of the compound (I) synthesized by the aforementioned production method

(5-2) Replacement of the substituent with a hydroxyl group, alkoxy group, etc.



Method J

The symbols in the formulae have the same meaning as defined above.

Process J1

The compound (XXIII) can be produced from the compound (XXII) by a demethylation reaction based on the reaction conditions according to the literature, such as Bull. Chem. Soc. Jpn., 44, 1986(1971); Org. Synth., Collect. Vol. V, 412(1073); J. Am. Chem. Soc., 78, 1380(1956); or J. Org. Chem., 42, 2761(1977). Examples of the reagent used for the demethylation reaction include 47% aqueous hydrobromic acid solution, boron tribromide, pyridine hydrochloride, and iodotrimethylsilane.

Process J2

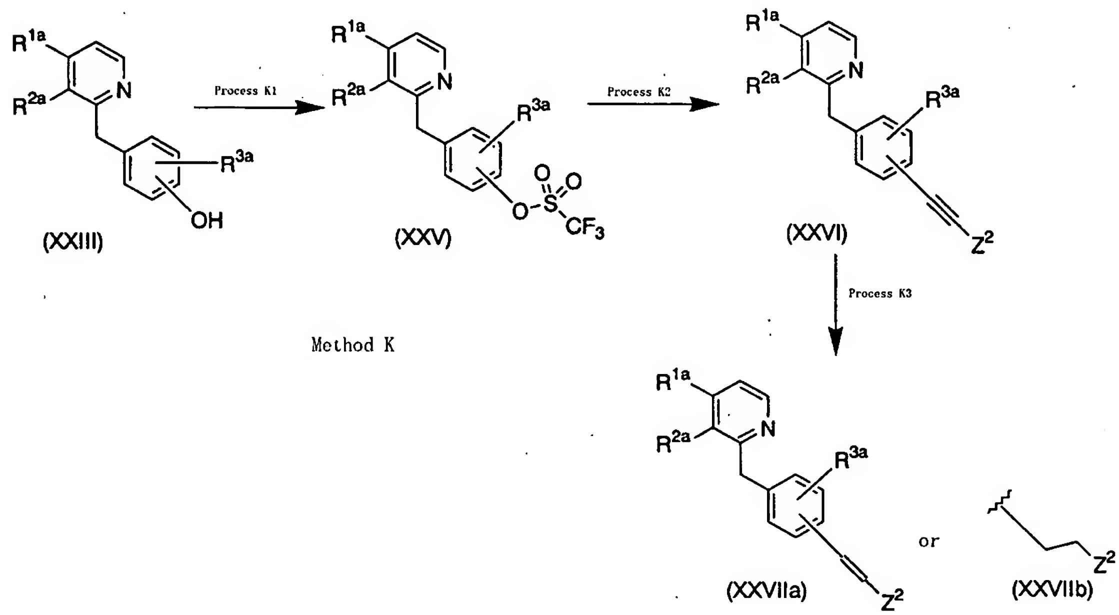
A process for the alkylation reaction. The compound (XXIV) can be produced by reacting the compound (XXIII) with a substituted or unsubstituted alkyl halide, a substituted or unsubstituted alkylmethane sulfonate, or such in the presence of a base.

Method J is a method for producing the compound (XXIV) via Process J1 and Process J2.

Production method (5-3)

Replacement of the substituent R^{3a} or R^{4a} of the compound (I) synthesized by the aforementioned production method

(5-3) Replacement of the substituent with a vinylene group, an ethynylene group, alkyl group, etc.



The symbols in the formulae have the same meaning as defined above.

Process K1

A process for the triflation reaction. The compound (XXV) can be produced by reacting the compound (XXIII) with trifluoromethane sulfonic acid anhydride in the presence of a base.

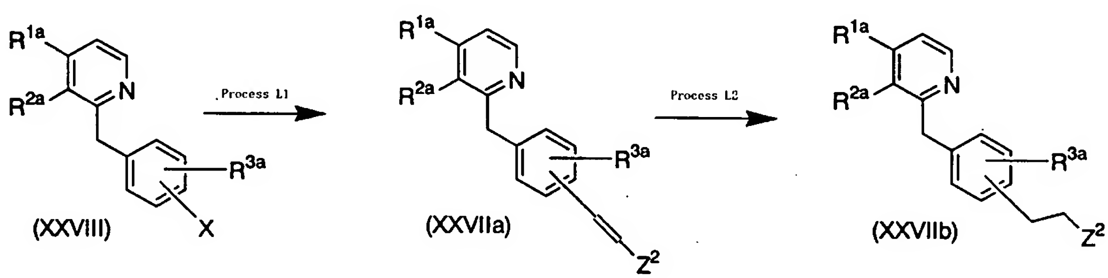
Process K2

A process for the coupling reaction with an alkyne. The compound (XXVI) can be produced by coupling the compound (XXV) with an alkyne derivative in the presence of a palladium phosphine complex, copper iodide, and a base. Examples of reagents that produce the palladium phosphine complex in the reaction system include a combination of palladium-carbon and triphenylphosphine, tetrakistriphenylphosphine palladium (0) and triphenylphosphine, dichlorobistriphenylphosphine palladium (II), palladium (II) acetate and tri(*o*-tolyl)phosphine, and

palladium(II) acetate and 1,1'-bis(diphenylphosphino)ferrocene. Examples of the base include triethylamine, piperidine, pyridine, and potassium carbonate. Depending on the reaction, lithium chloride may be used.

Process K3

A process for the reduction reaction of the unsaturated hydrocarbon. The compound (XXVIIa) or the compound (XXVIIb) can be produced from the compound (XXVI), for example, by catalytic hydrogenation using a catalyst. Examples of the catalyst include palladium-carbon, palladium hydroxide, platinum oxide, and palladium-carbon-calcium carbonate.



X denotes a leaving group, such as a halogen group and trifluorosulfonate.

Method L

The symbols in the formulae have the same meaning as defined above.

Process L1

A process of the coupling reaction (Heck Reaction) with the alkene. The compound (XXVIIa) can be produced from the compound (XXVIII) using a catalyst (e.g. palladium complex and its ligand), based on the reaction conditions according to the literature, such as J. Org. Chem., 37, 2320(1972); Org. Reactions., 27, 345(1982); Comprehensive Organic Synthesis, Vol. 4, 833(1991); Palladium Reagents and Catalysts, 125(1995); Chem. Commun., 1287(1984); Tetrahedron Lett, 26, 2667(1985); and Tetrahedron Lett, 31, 2463(1990). Examples of the combination of the catalysts used for this reaction (palladium complex and its ligand) include palladium (II) acetate and 1,1'-bis(diphenylphosphino)ferrocene, and palladium (II) acetate and

tri(*o*-tolyl)phosphine. Examples of the tertiary base include triethylamine, diisopropylethylamine, and 1,8-diazabicyclo[5.4.0]-7-undecene. X of the compound (XXVIII) denotes a leaving group, such as a halogen group and trifluoromethanesulfonyloxy group.

Process L2

The compound (XXVIIb) can be produced from the compound (XXVIIa) according to the conditions for a reduction reaction of an unsaturated hydrocarbon, similar to that of process K3.

Method L is a method for producing the compound (XXVIIa) by Process L1, followed by producing the compound (XXVIIb) by Process L2.

Various isomers of the compounds represented by the formula (I) of the present invention can be purified and isolated using ordinary separation techniques (for example, recrystallization, chromatography, and so on).

Compounds of the present invention or salts thereof, or hydrates thereof can be administered as they are to mammals (preferably humans). They can also be formulated by a conventional method into tablets, powders, fine granules, granules, coated tablets, capsules, syrups, troches, inhalants, suppositories, injections, ointments, eye ointments, eye drops, nasal drops, ear drops, cataplasms, lotions, and such, then administered. For the pharmaceutical formulation, ordinarily used auxiliary agents for pharmaceutical formulation (for example, fillers, binders, lubricants, coloring agents, flavoring agents, and as necessary, stabilizers, emulsifiers, absorbefacient, surfactants, pH regulators, antiseptics, antioxidants, etc.) can be used. The pharmatical formulation can be prepared by an ordinary method by combining components that are generally used as ingredients for pharmaceutical preparations. For example, oral preparations can be produced by combining the compounds of the present invention or a pharmaceutically acceptable salt thereof with fillers, and as necessary, binders, disintegrators, lubricants, coloring agents, flavoring agents, and such, and formulating the mixture into powders, fine granules,

granules, tablets, coated tablets, capsules, and such by usual methods. Examples of these components include animal fat and vegetable oil such as soybean oil, beef tallow, and synthetic glyceride; hydrocarbons such as liquid paraffin, squalene, and solid paraffin; ester oils such as octyldodecyl myristate and isopropyl myristate; higher alcohols such as cetostearyl alcohol and behenyl alcohol; silicone resin; silicone oil; surfactants such as polyoxyethylene fatty acid ester, sorbitan fatty acid ester, glycerol fatty acid ester, polyoxyethylene sorbitan fatty acid ester, polyoxyethylene hardened castor oil, and polyoxyethylene polyoxypropylene block copolymer; water-soluble macromolecules such as hydroxyethyl cellulose, polyacrylic acid, carboxyvinyl polymer, polyethylene glycol, polyvinyl pyrrolidone, and methyl cellulose; lower alcohols such as ethanol and isopropanol; polyhydric alcohols such as glycerol, propylene glycol, dipropylene glycol, and sorbitol; sugars such as glucose and sucrose; inorganic powder such as silicic acid anhydride, magnesium aluminum silicate, and aluminum silicate; purified water, etc. Examples of fillers include lactose, corn starch, refined white sugar, glucose, mannitol, sorbitol, crystalline cellulose, and silicon dioxide. Examples of binders include polyvinyl alcohol, polyvinyl ether, methyl cellulose, ethyl cellulose, gum arabic, tragacanth, gelatin, shellac, hydroxypropylmethyl cellulose, hydroxypropyl cellulose, polyvinyl pyrrolidone, polypropyleneglycol polyoxyethylene block polymer, and meglumine. Examples of disintegrators include starch, agar, powdered gelatin, crystalline cellulose, calcium carbonate, sodium hydrogencarbonate, calcium citrate, dextrin, pectin, and calcium carboxymethylcellulose. Examples of lubricants include magnesium stearate, talc, polyethyleneglycol, silica, and hardened vegetable oil. Examples of coloring agents are those accepted for addition to medicaments . Examples of flavoring agents include cocoa powder, *l*-menthol, aromatic dispersant, mint oil, borneol, and cinnamon powder. The use of sugar coating and other appropriate coating as necessary is of course permissible for these tablets and granules. Furthermore,

liquid preparations such as syrups and injections can be prepared using conventional methods by adding pH regulators, solubilizers, isotonizing agents, and such, and as necessary, solubilizing adjuvants, stabilizers, and such to the compounds of this invention or pharmaceutically acceptable salts thereof. The method for producing external preparations is not limited and can be produced by a conventional method. That is, base materials used for formulation can be selected from various materials ordinarily used for medicaments, quasi-drugs, cosmetics, and such. Specifically, the base materials to be used are, for example, animal fat and vegetable oil, mineral oil, ester oil, waxes, higher alcohols, fatty acids, silicone oil, surfactants, phospholipids, alcohols, polyhydric alcohols, water soluble macromolecules, clay minerals, and purified water. As necessary, pH regulators, antioxidants, chelating agents, antiseptic and antifungal agents, coloring matters, fragrances, and such may be added, but the base materials of the external preparations of the present invention are not to be construed as being limited thereto. Furthermore, as necessary, components such as those that have a differentiation induction effect, blood flow accelerants, fungicides, antiphlogistic agents, cell activators, vitamins, amino acids, humectants, and keratolytic agents can be combined. The above-mentioned base materials is added to an amount that leads to the concentration usually used for external preparations.

When the compounds of this invention or salts thereof, or hydrates thereof, is administered, there are no particular limitations on their form, and they can be administered orally or parenterally by a conventionally used method. They can be formulated into as dosage forms such as tablets, powder, fine granules, capsules, syrups, troches, inhalents, suppositories, injections, ointments, eye ointments, eye drops, nasal drops, ear drops, cataplasms, and lotions. The dose of the pharmaceutical compositions of this invention can be selected appropriately depending on the degree of the symptom, age, sex, weight, the dosage form, the type of salt, the specific type of disease, and

such.

A curative dose of the antifungal agent of this invention is administered to a patient. Herein, "curative dose" refers to the amount of the pharmaceutical agent that yields the desired pharmacological result and is effective for recovery or relief from the symptoms of a patient to be treated. The dose differs markedly depending on the weight of the patient, type of disease, degree of symptom, age of the patient, sex, sensitivity towards the agent, and such. Usually, the daily dose for an adult is approximately 0.03 to 1000 mg, preferably 0.1 to 500 mg, more preferably 0.1 to 100 mg, and is administered once to several times per day, or once to several times per several days. The dose for injections is normally, approximately 1 to 3000 μg/kg, and is preferably approximately 3 to 1000 μg/kg.

Brief Description of the Drawings

Fig. 1 is a schematic diagram of the process that transports GPI-anchored proteins to the cell wall. A GPI (Glycosylphosphatidylinositol)-anchored protein is first anchored to GPI, and then transported to the cell wall.

Fig. 2 is a graph showing the activity of the aforementioned compound (Ia) in the *S. cerevisiae* reporter system. In the presence of the aforementioned compound (Ia) at a concentration of 0.39 to 1.56 μg/ml, cephalosporinase activity increased in the culture supernatant fraction and decreased in the cell wall fraction, and at a concentration of 3.13 μg/ml or more, growth inhibition was observed.

Fig. 3 is a graph showing the effect of the aforementioned compound (Ia) on the adhesion of *C. albicans* to animal cells. Even at a concentration of 1.56 μg/ml in which growth inhibition cannot be observed, adhesion of *C. albicans* to animal cells was inhibited to about a half.

Fig. 4 is a graph showing the effect of the aforementioned compound (Ia) on the amount of the Als1p antigen of *C. albicans*. In the presence of the aforementioned compound (Ia) at a concentration

of 0.1 to 0.39 μg/ml, the amount of the Als1p antigen increased in the culture supernatant fraction and the amount of the antigen decreased in the cell wall fraction.

Fig. 5 is a photograph showing the Southern Blot analysis of the *C. albicans* gene using the GWT1 gene as a probe. A single band was observed at 6.5 kb with EcoRI, at 4.0 kb with HindIII, at 2.0 kb with EcoRI-HindIII, and at 2.5 kb with EcoRI-PstI, and the homologue of the resistant gene to the aforementioned compound (Ia) in *C. albicans* was expected to exist as a single gene.

Fig. 6 is a graph showing the activity of the aforementioned compound (Ia) in *S. cerevisiae* that overexpressed the GWT1 gene product. In *S. cerevisiae* CW63 strain ("W/T" in the Figure), even at the concentration of the aforementioned compound (Ia) (0.39 to 1.56 μg/ml) in which cephalosporinase activity in the culture supernatant fraction is increased, and activity in the cell wall fraction is decreased, such an effect was not observed in *S. cerevisiae* CW63/GWT1 strain, and in *S. cerevisiae* CW63 strain, even at the concentration of the aforementioned (> 3.13 μg/ml) in which growth is inhibited, growth inhibition was not observed in *S. cerevisiae* CW63/GWT1 strain ("O/E" in the Figure).

Fig. 7 is a diagram in which the highly conserved regions in the proteins encoded by the GWT1 genes of *S. cerevisiae*, *S. pombe*, and *C. albicans* are aligned.

Best Mode for Carrying out the Invention

[Example A]

The present invention is specifically illustrated below with reference to Examples, but it is not to be construed as being limited thereto.

Example A1 Construction of the reporter gene and introduction thereof into *S. cerevisiae*

(1). Construction of the reporter gene where lysozyme is the reporter

enzyme

A lysozyme gene comprising a promoter sequence was amplified by PCR using pESH plasmid comprising the ENO1 promoter + secretion signal + the lysozyme gene (Ichikawa K et al, Biosci. Biotech. Biochem., 57(10), 1686-1690, 1993) as template, and the oligonucleotides of SEQ ID NO: 8 and SEQ ID NO: 9 as primers, and this was subcloned into the *Sal*I-*Eco*RI site of pCR-Script SK(+) (a). Furthermore, a CWP2 gene was amplified by PCR using *S. cerevisiae* chromosomal DNA as template, and the oligonucleotides of SEQ ID NO: 10 and SEQ ID NO: 11 as primers, and this was subcloned into the *Eco*RI-*Hind*III site of pUC19 (b). Similarly, CYC1 terminator was amplified by PCR using pYES2 (INVITROGEN) as a template, and the oligonucleotides of SEQ ID NO: 12 and SEQ ID NO: 13 as primers, and this was subcloned into the newly introduced *Not*I-*Kpn*I site of pUC19 (c).

Next, the lysozyme gene excised with *Sal*I-*Eco*RI (a), and the CWP2 gene excised with *Eco*RI-*Hind*III (b) were inserted into the *Sal*I-*Hind*III cleavage site of pESH. Finally, pRLW63T was produced by excising a gene comprising the ENO1 promoter + secretion signal + lysozyme gene + CWP2 gene using *Bam*HI-*Hind*III, inserting this into a pRS306 integration vector (Sikorski RS et al, Genetics. 122(1):19-27, 1989), and then inserting the CYC1 terminator excised with *Hind*III-*Kpn*I (c) into the *Hind*III-*Kpn*I cleavage site.

(2). Construction of the reporter gene where cephalosporinase is the reporter enzyme

DNA comprising a promoter sequence and secretion signal portion was amplified by PCR using the abovementioned pESH as template, the ENO1 promoter C-terminus + secretion signal portion (d) as template, and the oligonucleotides of SEQ ID NO: 14 and SEQ ID NO: 15 as primers, and this was subcloned into the *Bam*HI-*Not*I site newly introduced into pUC19 (d). Furthermore, a cephalosporinase gene was amplified by PCR using *Citrobacter freundii* chromosomal DNA as template, and the oligonucleotides of SEQ ID NO: 16 and SEQ ID NO: 17 as primers, and this was subcloned into the *Nsp*V-*Xba*I site newly introduced into pUC19 (e).

Similarly, the CWP2 gene was amplified by PCR using the *S. cerevisiae* chromosomal DNA as template, and the oligonucleotides of SEQ ID NO: 18 and SEQ ID NO: 19 as primers, and this was subcloned into the *Xba*I-*Hind*III site of pUC19 (f).

After producing the full length ENO1 promoter + secretion signal portion by inserting the *Bam*HI-*Sal*I fragment of pESH into the *Bam*HI-*Sal*I cleavage site of a plasmid into which (d) has been inserted, the cephalosporinase gene excised with *Nsp*V-*Xba*I, and the CWP2 gene excised with *Xba*I-*Hind*III were inserted into the *Nsp*V-*Hind*III cleavage site. Next, pRCW63T was produced by excising with *Eco*RI-*Hind*III, inserting this fragment into the abovementioned pRS306, and then inserting the CYC1 terminator into the *Hind*III-*Kpn*I cleavage site.

(3). Introduction of the reporter gene into *S. cerevisiae*

S. cerevisiae G2-10 strain was cultured by shaking in 10 ml of YPD medium at 30°C, then the cells were collected at the late logarithmic growth phase (2-5x 10^7 cells/ml). After washing with sterilized water, the above mentioned pRLW63T and pRCW63T were introduced by lithium acetate method that uses YEASTMAKER™ Yeast Transformation System (Clontech) (according to the YEASTMAKER™ Yeast Transformation System User Manual). pRLW63T and pRCW63T in which the URA3 gene was cleaved with *Eco*RV and *Apa*I, respectively, were used. After culturing in SD(Ura^-) medium at 30°C for 3 days, the grown colonies were cultured in YPD medium.

When the localizations of lysozyme and cephalosporinase activities were confirmed, both activities were mainly localized in the cell wall, and the C-terminal sequence of CWP2 was confirmed to function as a transport signal to the cell wall.

Example A2 Screening of pharmaceutical agents by the *S. cerevisiae* reporter system

Since sensitivity of the enzyme reaction is better with cephalosporinase compared to lysozyme, *S. cerevisiae* introduced with pRCW63T (*S. cerevisiae* CW63 strain) was used for the screening of

compounds.

After stationary cultivation in YPD liquid medium at 30°C for 48 hours, the yeast cell culture was diluted 100 times with YPD liquid medium (3-5x 10^5 cells/ml) and 75 µl/well aliquots thereof were inoculated into a V-bottomed 96-well plate containing 25 µl/well of a diluted test sample, and this was subjected to stationary cultivation at 30°C for 48 hours. After centrifuging the plate, 25 µl of the supernatant was sampled and placed in a flat-bottomed 96-well plate, and this was used as the culture supernatant fraction.

The precipitated cells were suspended, and 75 µl/well aliquots of Zymolyase (Seikagaku Corporation) solution prepared with 2.4 M sorbitol were added and were allowed to react at 30°C for 1 hour. After centrifuging the plate, 10 µl of the supernatant was sampled and placed in a flat-bottomed 96-well plate, 15 µl of phosphate buffer was added, and this was used as the cell wall fraction.

The cephalosporinase activities in the medium and in the cell wall fraction were measured by adding 200 µM of nitrocefin solution to a pooled sample, and after a certain period of time, stopping the reaction with citric acid buffer, and then measuring the absorbance at 490 nm.

Furthermore, fungal growth in the presence of the test sample was determined by visual observation.

Fig. 2 showed that in the presence of the aforementioned compound (Ia) at a concentration of 0.39 to 1.56 µg/ml, cephalosporinase activity increases in the culture supernatant fraction, and the activity decreases in the cell wall fraction. In this manner, a compound that increases the cephalosporinase activity in the culture supernatant fraction, and in addition decreases the cephalosporinase activity in the cell wall fraction was considered to be a compound that inhibits the process that transports GPI-anchored proteins to the cell wall.

Example A3: Screening of pharmaceutical agents using the adhesion of *Candida* to animal cells

Three-milliliter aliquots of IEC-18 cells (1x 10^5 cells/ml in D-MEM

medium (Nissui Pharmaceutical) containing 10% fetal calf serum and 2 mM glutamine) were placed in each well of a 6-well multi-well plate. The plate was incubated in a carbon dioxide gas incubator at 37°C for 3 days, the culture supernatant was removed, and ethanol immobilization was carried out.

C. albicans cultured in Sabouraud Dextrose Liquid Medium containing various concentrations of the test sample at 30°C for 48 hours was adjusted to 4x 10^2 cells/ml, and 1 ml was inoculated into each well of the plate in which the immobilized IEC-18 cells were cultured. After cultivation at 30°C for 1 hour, the culture supernatant was removed, washed with PBS, and then 2 ml of Sabouraud Dextrose Agar Medium (Difco) was superposed. After cultivation at 30°C overnight, the number of colonies (CFU) that had grown was counted and the adhesion rate was calculated.

Fig. 3 shows that even at a concentration of 1.56 μg/ml of the aforementioned compound (Ia), in which growth inhibition cannot be observed, adhesion of *C. albicans* to animal cells was inhibited to about a half. Compared to untreated *C. albicans*, a test sample that diminished CFU that adhered to cells was considered as a compound that inhibits the adhesion of *C. albicans* to animal cells.

Example A4: Screening of pharmaceutical agents using the amount of the GPI-anchored protein quantified by ELISA

(1). Production of anti-Als1p peptide antibody

A house rabbit was immunized with the synthetic peptide of SEQ ID NO: 20 which was conjugated with KLH. The obtained antisera was affinity-purified, and the IgG fraction was used as the anti-Als1p peptide antibody.

(2). Screening of pharmaceutical agents by ELISA using anti-Als1p peptide antibody

C. albicans was cultured in Sabouraud Dextrose Liquid Medium (5 ml) containing various concentrations of the test sample at 30°C for 48 hours, and the cells were collected by centrifugation, washed, and

then suspended in 300 μl of Tris-HCl buffer. The suspended cells were transferred to a microtube containing glass beads, and were disrupted by repeating 10 cycles of stirring for 1 minute and cooling on ice for 1 minute. The disrupted cells that were washed were extracted with 2% SDS at 95°C for 10 minutes, centrifuged, and then the precipitate was washed 5 times with phosphate buffer. To this precipitate, 0.5 ml of 5 μg/ml Zymolyase solution was added, reacted at 37°C for 1 hour, and the centrifuged supernatant was used as the GPI-anchored protein sample.

A 96-well plate was coated with 50 μl of anti-Als1p peptide antibody (40 μg/ml) at 4°C overnight. After washing 5 times with PBS containing 0.05% Tween 20 (PBST), blocking was carried out with 25% BlockAce at room temperature for 2 hours. After washing 3 times with PBST, 50 μl of the 2-fold serially diluted GPI-anchored protein sample was reacted at room temperature for 2 hours. After washing 5 times with PBST, 100 μl of 1000-fold diluted HRP-labeled anti-*Candida* antibody (ViroStat) was reacted at room temperature for 2 hours, then upon washing 5 times with PBST, 75 μl of substrate solution was added. After the reaction was stopped, absorbance at 490 nm was measured.

Fig. 4 shows that in the presence of the aforementioned compound (Ia) at a concentration of 0.1 to 0.39 μg/ml, the amount of Als1p antigen increases in the culture supernatant fraction, and the amount of antigen decreases in the cell wall fraction. In this manner, a compound that increased the amount of Als1p in the culture supernatant, or decreased the amount of Als1p in the cell wall fraction, as quantified by ELISA, compared to the amount of Als1p in *C. albicans* untreated with the compound, was considered to be a compound that inhibits the process that transports GPI-anchored proteins to the cell wall in *C. albicans*.

Example A5 Observation of the cell wall of *C. albicans* cultured in the presence of a test sample by an electron microscope

C. albicans which was cultured in Sabouraud Dextrose Liquid Medium (5 ml) containing various concentrations of the test agent at 30°C for 48 hours, then centrifuged, and collected, was immobilized by potassium

permanganate immobilization method, and the transmission electron microscope image thereof was observed.

The flocculent fibrous structure with high electron density was observed in the outermost layer of the cell, and was considered to be the surface layer glycoprotein layer having the GPI-anchored protein as its constituent. This flocculent fibrous structure was not influenced by other existing antifungal agents.

In *C. albicans* cultured in the presence of the aforementioned compound (Ia), the flocculent fibrous structure of the outermost layer of the cell having high electron density disappeared leaving a small amount of the layer with high electron density, compared to that in untreated cells. In this manner, when the flocculent fibrous structure of the outermost layer of the fungal cell having high electron density disappeared, the test sample was considered to be the compound influencing the process that transports GPI-anchored proteins to the cell wall.

Example A6: Screening of the resistant gene to the aforementioned compound (Ia) of *S. cerevisiae*

The plasmid library of the *S. cerevisiae* gene was obtained from ATCC (Information for ATCC Number: 37323).

S. cerevisiae G2-10 strain was cultured while shaking in 10 ml of YPD medium at 30°C, and cells were collected at the late logarithmic growth phase (1-2x 10^7 cells/ml). After washing the cells with sterilized water, the plasmid library of the *S. cerevisiae* gene was introduced by the lithium acetate method that uses YEASTMAKER™ Yeast Transformation System (Clontech) (according to YEASTMAKER™ Yeast Transformation System User Manual), and this was spread onto a SD(Leu^-) plate, and approximately 80,000 colonies were obtained. The colonies were collected and diluted, and were spread onto a SD(Leu^-) plate containing the aforementioned compound (Ia) at a concentration of 1.56 μg/ml and 3.125 μg/ml so that there were 570,000 colonies per plate. Subsequently, the resistant clone was obtained by incubation at 37°C

for 72 hours.

When 27 clones were picked and plasmids were collected by the method according to METHODS IN ENZYMOLOGY, Vol. 194: 169-182 (1991), and the inserts were analyzed, all 27 contained the same fragment.

As a result of determining the nucleotide sequence using the ABI377 system (PE Applied Biosystems), the DNA of SEQ ID NO: 1 was found to be the DNA that confers resistance to the aforementioned compound (Ia), and was named GWT1.

Example A7: Southern Blot analysis of a *C. albicans* homologue of the *S. cerevisiae* GWT1 gene.

A sample was prepared by treating 25 µg of the *C. albicans* genomic DNA with *Eco*RI (TaKaRa), *Hind*III (TaKaRa), *Bam*HI (TOYOBO), or *Pst*I (New England Biolabs) (including a combination of 2 types of enzymes) for 16 hours, then concentrating by ethanol precipitation, and dissolving in 25 µl of sterilized water. Twenty-five micrograms of genomic DNA digested with restriction enzymes was separated by 0.75% agarose gel electrophoresis method, and was transferred to a nylon membrane (GeneScreen PLUS /NEN).

A probe was produced by labeling 20 ng of the approximately 1.5 kb DNA fragment of SEQ ID NO: 1 with alpha33P-dCTP by the random primer method, and was purified using a GeneQuant column (Amersham-Pharmacia).

Hybridization was carried out by soaking the membrane in 10 ml of PerfectHyb™ (TOYOBO) solution, preincubating at 65°C for 1 hour, then adding the labeled probe mentioned above, and incubating at 65°C for 2.5 hours. Washing was carried out with 1). 2x SSC, 0.05% SDS solution at 25°C for 5 minutes, 2). 2x SSC, 0.05% SDS solution at 25°C for 15 minutes, and 3). 0.1x SSC, 0.1% SDS solution at 50°C for 20 minutes. The washed membrane was wrapped with Saran Wrap, and contacted with an Imaging Plate (FUJI) for 12 hours at room temperature, the image that was transferred to the Imaging Plate was captured using BAS2000 (FUJI), and the image was analyzed.

As a result, single bands were observed at 6.5 kb with *Eco*RI, 4.0

kb with *Hind*III, 2.0 kb with *Eco*RI-*Hind*III, and 2.5 kb with *Eco*RI-*Pst*I (Figure 5), and the homologue of the resistant gene to the aforementioned compound (Ia) of *C. albicans* was expected to exist as a single gene.

Example A8: Screening of the resistant gene to the aforementioned compound (Ia) of *C. albicans*

The genomic library of *C. albicans* was produced by the method according to Navaro-Garcia F et al, Mol. Cell. Biol., 15: 2197-2206, 1995. Specifically, the genomic DNA of *C. albicans* was partially digested with *Sau*3AI, then DNA fragments around 3 to 5 were collected, and these were inserted into the *Bam*HI site of YEp352 shuttle vector.

S. cerevisiae G2-10 strain was cultured by shaking in 10 ml of YPD medium at 30°C, and cells were collected at the late logarithmic growth phase (2-5x 10^7 cells/ml). After washing the cells with sterilized water, a genomic library of the *C. albicans* was introduced by the lithium acetate method that uses YEASTMAKER™ Yeast Transformation System (Clontech) (according to YEASTMAKER™ Yeast Transformation System User Manual), and this was spread onto a SD(Ura^-) plate, and approximately 25,000 colonies were obtained. The colonies were collected and diluted, and were spread onto a SD plate containing the aforementioned compound (Ia) at a concentration of 1.56 μg/ml so that there were 500,000 colonies per plate. Subsequently, the resistant clones were obtained by incubation at 30°C for 6 hours, and then transferred to 37°C and incubated for 66 hours.

When 30 clones were picked and plasmids were collected by the method according to METHODS IN ENZYMOLOGY, Vol. 194: 169-182 (1991), and the inserts were analyzed, 28 out of 30 contained the same fragment.

As a result of determining the nucleotide sequence using the ABI377 system (PE Applied Biosystems), the DNA of SEQ ID NO: 3 was found to be the DNA that confers resistance to the aforementioned compound (Ia).

Example A9: Cloning of a homologue of the resistant gene to the aforementioned compound (Ia) from the clinical isolate of *C. albicans*.

PCR amplification was carried out using as template a genomic DNA that was purified from a clinical isolate of *C. albicans* that is stored by the inventors, and SEQ ID NO: 21 and SEQ ID NO: 22 as primers. A DNA fragment of approximately 1.6 kb was amplified from all three of the independent PCR samples, the amplified fragments were purified, subcloned into a pT7-Blue vector (Novagen), and the nucleotide sequence was determined, and thereby, the DNA sequence of SEQ ID NO: 5 was discovered. The sequence was different at three positions as compared to the DNA of Example A7 (SEQ ID NO: 3).

Furthermore, in the nucleotide sequence of the *C. albicans* gene determined at Stanford University Sequence Center (http://sequence-www.stanford.edu/), a homologue of the DNA of Example A7 was found (SEQ ID NO: 7), and the sequence was different at four positions as compared to the DNA of Example A7 (SEQ ID NO: 3).

Example A10: Construction of *S. cerevisiae* overexpressing the GWT1 gene product

PCR amplification was carried out using a plasmid purified from the resistant clone to the aforementioned compound (Ia) obtained in Example A6 as a template, and SEQ ID NO: 23 and SEQ ID NO: 24 as primers. A PCR product cleaved with *Pvu*II was inserted into the *Sal*I-*Hind*III cleavage site of pRLW63T produced in Example A1. The entire insert was excised with *Bam*HI-*Kpn*I, and was inserted into the MCS (multi-cloning site) of pRS304 (Sikorski RS et al, Genetics. 122(1): 19-27, 1989) to produce a vector for integration.

S. cerevisiae CW63 strain having a cephalosporinase gene as the reporter gene was cultured by the method according to Example A1, TRP1 of the integration vector was cleaved with *Eco*RV, and then transformation was carried out by the method of Example A1. GWT1-overexpressed strain (*S. cerevisiae* CW63/GWT1 strain) was obtained by culturing in SD(Trp^-) medium at 30°C for 3 days.

Other than showing resistance to the aforementioned compound (Ia), GWT1-overexpressed strain is not different from the wild type strain,

and was sensitive towards other antifungal agents, cycloheximide, benomyl, and amphotericin B.

Example A11: Construction of *S. cerevisiae* mutant lacking the GWT1 gene

His5 cassette containing the GWT1 sequence on both ends was amplified by PCR using the his5 gene of *S. pombe* (Longtine MS et al, Yeast, 14: 953-961, 1998) as template and SEQ ID NO: 25 and SEQ ID NO: 26 as primers.

S. cerevisiae G2-10 was cultured and the cells were collected by the method according to Example A1, and the abovementioned PCR product was transformed by the method according to Example A1. A GWT1-deficient strain was obtained by cultivation in SD(His^-) medium at 30°C for 5 to 7 days.

Although the GWT1-deficient strain shows very slow growth, it was suggested that the growth is not influenced by the aforementioned compound (Ia), and the GWT1 gene product is the target of the compound. Furthermore, the GWT1-deficient strain indicated the following characteristics: it cannot grow at high temperatures; the cells are swollen; and in the observation by a transmission electron microscope, the flocculent fibrous structure of the outermost layer of the fungal cell having high electron density had disappeared.

Example A12: Activity of the aforementioned compound (Ia) in *S. cerevisiae* overexpressing the GWT1 gene product

Using *S. cerevisiae* CW63 strain and GWT1 gene introduced *S. cerevisiae* CW63/GWT1, activity of the aforementioned compound (Ia) was examined by a method according to the method described in Example A2.

As a result, even at a concentration (0.39 to 1.56 μg/ml) of the aforementioned compound (Ia) at which cephalosporinase activity in the culture supernatant fraction is increased, and the activity in the cell wall fraction is decreased in *S. cerevisiae* CW63 strain, no influence was observed in the *S. cerevisiae* CW63/GWT1 strain, and even at a concentration (> 3.13 μg/ml) of the aforementioned compound (Ia) at which

growth is inhibited in *S. cerevisiae* CW63 strain, growth inhibition was not observed in the *S. cerevisiae* CW63/GWT1 strain (Fig. 6).

Example A13: Synthesis of (4-butylphenyl)(1-isoquinolyl)ketone

Under a nitrogen atmosphere, 1-bromo-4-butylbenzene (2.29 ml, 13.0 mmol) was added to a mixed solution of magnesium (338 mg, 13.9 mmol) and tetrahydrofuran (6.5 ml), and as an initiator, catalytic amount of 1,2-dibromoethane was added, and this was stirred under reflux for 10 minutes. The solution was cooled to 0°C, a tetrahydrofuran solution of 1-isoquinolinecarbonitrile (1.0g, 6.49 mmol) was added, and was stirred for another 1 hour at room temperature, and at 70°C for 3 hours. Subsequently, the solution was cooled again to 0°C, concentrated hydrochloric acid (2.56 ml) and methanol (11 ml) were added, and then refluxed for 2 hours. The concentrated residue was dissolved in 5 N sodium hydroxide and toluene, and was filtered through celite. The toluene layer of the filtrate was divided, washed with water, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography to give 1.72 g of the title compound.

^{1}H-NMR($CDCl_3$) δ (ppm):0.93(3H, t), 1.32-1.43(2H, m), 1.58-1.66(2H, m), 2.68(2H, t), 7.28(2H, d), 7.61(1H, td), 7.74(1H, td), 7.80(1H, d), 7.87(2H, d), 7.92(1H, d), 8.20(1H, d), 8.60(1H, d)

Example A14 Synthesis of {1-(4-butylbenzyl)isoquinoline}, the aforementioned compound of the formula (Ia)

The compound of Example A13 (1.72g, 5.95 mmol), hydrazine monohydrate (836 mg, 16.7 mmol), and potassium hydroxide (769 mg, 13.7 mmol) were added to diethylene glycol (8.5 ml), and were stirred at 80°C for 1 hour, at 160°C for 3 and a half hours, and at 200°C for 1 hour. Upon cooling to room temperature, ice water was added and extracted with ethyl acetate. This was washed with water, then dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography to give 914 mg of the aforementioned compound of the formula (Ia).

^1H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.50-1.59(2H, m), 2.53(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.53(1H, td), 7.56(1H, d), 7.64(1H, td), 7.81(1H, d), 8.18(1H, dd,), 8.50(1H, d)

Example A15: Another method for producing {1-(4-butylbenzyl)isoquinoline}, the aforementioned compound of the formula (Ia)

To a dimethylformamide (1.8 ml) solution of 60% sodium hydride (16 mg, 0.40 mmol), a dimethylformamide (3.6 ml) solution of 1-cyano-2-benzoyl-1,2-dihydroisoquinoline (100 mg, 0.38 mmol) synthesized according to the literature of Org.Synth.,VI,115(1988), and 4-*n*-butylbenzylchloride (70 mg, 0.38 mmol) was added dropwise under nitrogen atmosphere at -16°C, and was further stirred at room temperature for 30 minutes. Water was added, this was concentrated, and toluene and water were added to this residue. The toluene layer was washed with water, dried over potassium carbonate, and concentrated. To an ethanol (1.6 ml) solution of the residue, 50% aqueous sodium hydroxide solution (0.63 ml) was added, and this was refluxed for 2 hours. After concentration, toluene and water were added. The toluene layer was washed with water, then dried over calcium carbonate, and then concentrated. The residue was purified by silica gel column chromatography to give 18 mg of the aforementioned compound of the formula (Ia).

Example A16 Cloning of the *C. albicans* homologue of the *S. cerevisiae* GWT1 gene

The *C. albicans* genomic DNA (25 µg) treated with *Hind*III (TaKaRa) for 16 hours was separated by 0.75% agarose gel electrophoresis method, and the DNA fragments ranging in size from approximately 3.5 to 4.5 kb were recovered from the gel. The recovered DNA fragments were inserted into the *Hind*III site of the pKF3 vector (TaKaRa), and a *Candida* genomic library was produced.

Using the produced library, approximately 10,000 colonies were

displayed on an LB/Ampicillin plate, colony lifting was performed using a Colony/Plaque Screen (NEN) membrane, and then this was subjected to hybridization. A probe was produced by labeling 20 ng of the approximately 1.5 kb DNA fragment of SEQ ID NO: 1 with alpha ^{33}P-dCTP by the random primer method, and purifying using a GeneQuant column (Amersham-Pharmacia).

Hybridization was carried out by pre-incubating the membrane in a PerfectHyb™ (TOYOBO) solution at 65°C for 1 hour, then adding the labeled probe mentioned above, and incubating further at 65°C for 2.5 hours. Washing was carried out with (i) 2x SSC, 0.05% SDS solution at 25°C for 5 minutes, (ii) 2x SSC, 0.05% SDS solution at 25°C for 15 minutes, and (iii) 0.1x SSC, 0.1% SDS solution at 50°C for 20 minutes. The washed membrane was wrapped with Saran Wrap, contacted with an X-RAY FILM (KONICA) for 24 hours at room temperature, and then developed. The *E. coli* colonies corresponding to the exposed spots were isolated, and were subjected to secondary screening. Approximately 200 of the isolated colonies were displayed on each LB/Ampicillin plate, colony lifting was performed in a similar manner to primary screening, which was followed by hybridization. The conditions for hybridization were the same as the conditions for primary screening.

As a result, a single colony of *E. coli* that reacts strongly with the probe was isolated. Plasmids were collected from this colony, and when the contained sequence was determined, a novel sequence having the same sequence as that revealed in Example A9 (SEQ ID NO: 5) was found (the sequence of *Candida* GWT1), and was presumed to be a *C. albicans* homologue.

Example A17: The *S. Pombe* homologue of the *S. cerevisiae* GWT1 gene

S. Pombe genes that show homology to the *S. cerevisiae* GWT1 gene (SEQ ID NO: 27, and the amino acid sequence of the gene product thereof: SEQ ID NO: 28) were found from a database search, and were considered to be the *S. Pombe* homologues of GWT1.

Example A18: Cloning of the *Aspergillus fumigatus* homologue of the *S. cerevisiae* GWT1 gene

By genetic sequence analysis, the inventors discovered two highly conserved regions in the protein encoded by the GWT1 genes of *S. cerevisiae*, *S. pombe*, and *C. albicans* (Fig. 7). Based on the presumed DNA that encodes the amino acid sequence of this conserved region, primers of SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31 were designed. PCR amplification was carried out using 1 μl of the library purchased from STRATAGENE (*Aspergillus fumigatus* cDNA library: #937053) as a template, and using primers of SEQ ID NO: 29 and SEQ ID NO: 31. Furthermore, as a result of carrying out nested-PCR using 1 μg of this amplified sample as a template, and using primers of SEQ ID NO: 29 and SEQ ID NO: 30, amplification of a single fragment of approximately 250 bp was confirmed. When the sequence of this fragment was determined, a novel sequence having homology to the GWT1 gene of *S. cerevisiae*, shown in SEQ ID NO: 32, was obtained, and this was presumed to be the homologue of *A. fumigatus*.

To obtain a full length cDNA, primers of SEQ ID NO: 33 and SEQ ID NO: 34 were designed based on the sequence of the amplified fragment. Furthermore, primers outside the gene insertion site of the library, SEQ ID NO: 35 and SEQ ID NO: 36, were designed. As a result of performing PCR using the *A. fumigatus* cDNA library as a template, and the primer set of SEQ ID NO: 33 and SEQ ID NO: 35, or the primer set of SEQ ID NO: 34 and SEQ ID NO: 36, amplification of a DNA fragment of approximately 1 kb was confirmed (by both primer sets). As a result of determining the nucleotide sequences of these fragments, a novel sequence that is highly homologous to the GWT1 genes of *S. cerevisiae* shown in SEQ ID NO: 1 was obtained. Since the sequence is highly homologous to the GWT1 genes of *S. cerevisiae*, *S. pombe*, and *C. albicans* throughout the entire gene, this sequence was strongly suggested to be a homologue of *A. fumigatus*.

To clone the entire homologue of *A. fumigatus*, the primer shown in SEQ ID NO: 37 that corresponds to the sequence upstream of the

initiation codon, and the primer of SEQ ID NO: 38 that corresponds to the sequence downstream of the stop codon were newly designed based on the obtained sequence. As a result of performing 35 cycles of PCR using the *A. fumigatus* cDNA library (STRATAGENE) and the *A. fumigatus* genomic library (STRATAGENE) as templates, and primers of SEQ ID NO: 37 and SEQ ID NO: 38, a single amplified fragment of approximately 1.6 kb was detected from both templates. As a result of determining the nucleotide sequence of this fragment by Direct-Sequencing, the nucleotide sequence shown in SEQ ID NO: 39 was found from the cDNA library, and was suggested to encode a protein comprising 501 amino acids shown in SEQ ID NO: 40. Furthermore, the nucleotide sequence of SEQ ID NO: 41 was found from the genomic library, and was found to have an intron comprising 77 base pairs in one position.

Example A19: Cloning of the *Cryptococcus* homologue of the *S. cerevisiae* GWT1 gene

1). Database search

As a result of database searching for genes showing homology to the *S. cerevisiae* GWT1 gene, the sequence of 502042C05.x1 was found from the server of the Genome Center at Stanford University (http://baggage.stanford.edu/cgi-misc/cneoformans/). Furthermore, the sequence of b6e06cn.f1 was found from the server at Oklahoma University, U.S.A (http://www.genome.ou.edu/cneo_blast.html).

2). PCR using genomic DNA as template

The primer of SEQ ID NO: 42 was constructed based on the sequence of 502042C05.x1, and the primer of SEQ ID NO: 43 was constructed based on the sequence of b6e06cn.f1. When PCR amplification was carried out using the genomic DNA of *Cryptococcus* (*Cryptococcus neoformans*) as a template, and using the primer of SEQ ID NO: 42, and the primer of SEQ ID NO: 43, an amplified fragment of approximately 2 kb was detected. When the nucleotide sequence of this fragment was determined, a novel sequence showing homology to the GWT1 gene of *S. cerevisiae*, shown in SEQ ID NO: 44, was obtained.

In order to obtain the sequence upstream of the initiation codon of the *Cryptococcus* GWT1 gene, the primer of SEQ ID NO: 45 was designed based on the sequence of 502042C05.x1, and the primer of SEQ ID NO: 46 was designed based on the sequence of SEQ ID NO: 44. When PCR amplification was carried out using the genomic DNA of *Cryptococcus* as a template, and using the primer of SEQ ID NO: 45, and the primer of SEQ ID NO: 46, an amplified fragment of approximately 500 bp was detected. When the nucleotide sequence of this fragment was determined, the sequence of SEQ ID NO: 47 was obtained, and this was found to overlap with SEQ ID NO: 44.

3). 3'-RACE

To obtain the 3'-terminal sequence of the *Cryptococcus* GWT1 gene, 3'-RACE was carried out. Reverse transcription was carried out by priming with the adaptor-primer of SEQ ID NO: 48, which is based on 16 μg of total RNA extracted from *Cryptococcus*, and by using SuperScript II Reverse Transcriptase (GIBCO/BRL), and a single stranded cDNA, which is to become the template for the RT-PCR that follows, was produced. As a result of performing 35 cycles of PCR using the single stranded cDNA as a template, and the primers of SEQ ID NO: 49 and SEQ ID NO: 50, an amplified fragment of approximately 1.2 kb was detected. When the nucleotide sequence of this fragment was analyzed by the Direct-Sequencing method, the novel sequence shown in SEQ ID NO: 51 showing homology to the *S. cerevisiae* GWT1 gene was obtained.

4). PCR of a full length genomic DNA

Using the primer of SEQ ID NO: 52 that was designed based on SEQ ID NO: 47, and the primer of SEQ ID NO: 53 that was designed based on SEQ ID NO: 51, 35 cycles of PCR was carried out on three independent preparations with the genomic DNA of *Cryptococcus* as template. As a result, an amplified fragment of approximately 2 kb was detected from all three of the independent tubes, and therefore, each of them were individually subjected to Direct-Sequencing, and their entire nucleotide sequences were determined. As a result, the three independent sequences completely matched, and a sequence comprising the

full length GWT1 gene homologue of *Cryptococcus* shown in SEQ ID NO: 54 was obtained.

5). Determination of the cDNA sequence

Comparison of the sequence of the *Cryptococcus* GWT1 gene derived from the genome shown in SEQ ID NO: 54 with cDNA sequence 51 obtained by 3'-RACE suggested the presence introns at two positions. Furthermore, since the open reading frame following the ATG initiation codon is not continuous, the presence of another intron was suggested. Therefore, the cDNA structure was predicted from the presumed amino acid sequence and the splicing donor/acceptor sequence, and the primers of SEQ ID NO: 55 and SEQ ID NO: 56 were designed at the position predicted to be the junction between exons. As a result of performing 35 cycles of PCR using the single stranded cDNA derived from *Cryptococcus* as template with the above-mentioned primers, an amplified fragment of approximately 1.4 kb was confirmed. As a result of determining the nucleotide sequence by subjecting the fragment to Direct-Sequencing, the sequence of SEQ ID NO: 57 was obtained, and by comparing with SEQ ID NO: 54, the cDNA sequence of the GWT1 gene of *Cryptococcus* was suggested to have the structure of SEQ ID NO: 58. Since the sequence shows high homology at certain regions with the GWT1 genes of *S. cerevisiae*, *S. pombe*, *C. albicans*, and *A. fumigatus*, this sequence was strongly suggested to be a homologue of *Cryptococcus*.

Example A20: Genetic mutation that confers resistance to the aforementioned compound of the formula (Ia)

S. cerevisiae LW63 strain having a lysozyme gene as the reporter gene due to introduction of pRLW63T was treated with ethyl methanesulfonate, then by culturing in a SD medium containing the aforementioned compound of the formula (Ia) at concentrations of 1.56, 3.13, and 6.25 µg/ml at 37°C for 3 days, five resistant mutant strains (R1 to R5) were obtained. Among them, the R1 mutant strain and the R5 mutant strain were found to have acquired a specific resistant characteristic to the aforementioned compound of the formula (Ia) due

to a mutation of a single gene. To confirm whether or not these two mutant strains have mutations on the GWT1 gene, genomic DNAs were extracted from both mutant strains, and the nucleotide sequence of the GWT1 gene portion was determined. As a result, in the R1 mutant strain, guanine at position 1213 had been mutated to adenine. Furthermore, in the R5 mutant strain, guanine at position 418 had been mutated to adenine. Therefore, it was elucidated that in the R1 mutant strain, the 405th amino acid, isoleucine, had been changed to valine, and in the R5 mutant strain, the 140th amino acid, glycine, had been changed to arginine.

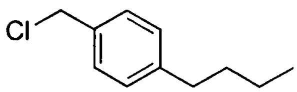
Next, to confirm whether or not these mutations are the cause of the acquisition of the specific resistant characteristic to the aforementioned compound of the formula (Ia), the mutant GWT1 gene (R1 or R5) was isolated using the genomic DNAs derived from both mutant strains as templates and the primers of SEQ ID NOS: 60 and 61. Simultaneously, the GWT1 promoter region (SEQ ID NO: 62) and the terminator region (SEQ ID NO: 63) were isolated, the GWT1 gene promoter, mutant GWT1 gene ORF, and the GWT1 gene terminator were inserted into the pRS316 vector, and plasmids that express a single copy of the mutant GWT1 gene were constructed (pRS316GWT1-R1, pRS316GWT1-R5). This was introduced to a diploid strain (WDG1) in which only a single copy of the GWT1 gene is disrupted. Spores were formed by culturing the colonies on a sporulation medium, and a clone in which the GWT1 gene on the chromosome is disrupted and also harbors the abovementioned plasmid was obtained by performing a tetrad analysis. When this was cultured in a medium containing the aforementioned compound of the formula (Ia), resistance to the aforementioned compound of the formula (Ia) was seen, similarly to the original R1 mutant strain and R5 mutant strain. From the above, it was elucidated that the specific resistant characteristic to the aforementioned compound of the formula (Ia) is conferred by a point mutation accompanying an amino acid mutation, that occurred on the GWT1 gene, and this compound was strongly suggested to inhibit the function of the GWT1 protein by directly binding to the protein.

[Example B]

The compounds of this invention can be produced, for example, by the method of the Examples below. However, the Examples are for illustration purpose only and the compounds of this invention are not to be construed as being limited to those prepared in the following specific examples under any circumstances.

Example B1

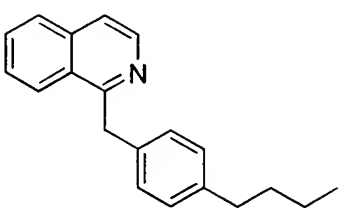
1-(Chloromethyl)-4-*n*-butylbenzene



Thionyl chloride (2.5 ml, 34 mmol) was added to a solution of 4-*n*-butylbenzyl alcohol (2.0 g, 12 mmol) in ether (25 ml), and this mixture was stirred at room temperature for 3 hours. After concentration of the mixture, excess thionyl chloride was removed by azeotropic distillation with benzene to give the title compound (2.3 g). This compound was used in the following reaction without purification.

Example B2

1-(4-Butylbenzyl)isoquinoline



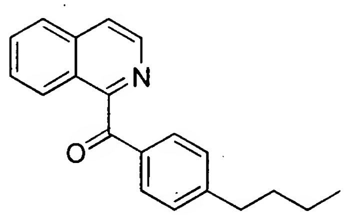
A solution of 1-cyano-2-benzoyl-1,2-dihydroisoquinoline (100 mg, 0.38 mmol), which was synthesized according to Org. Synth., VI, 115 (1988), and 4-*n*-butylbenzyl chloride (70 mg, 0.38 mmol) in dimethylformamide (3.6 ml) was added dropwise to a solution of 60% sodium hydride (16 mg, 0.40 mmol) in dimethylformamide (1.8 ml) under nitrogen atmosphere at -16°C, and this mixture was stirred at room temperature for 30 minutes. Water was added, the mixture was concentrated under

reduced pressure, and toluene and water were added to the residue. The toluene layer was washed with water, dried over potassium carbonate, then concentrated under reduced pressure. A 50% aqueous sodium hydroxide solution (0.63 ml) was added to a solution of the residue in ethanol (1.6 ml).This mixture was heated under reflux for 2 hours and concentrated, and then toluene and water were added. The toluene layer was washed with water, dried over calcium carbonate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (18 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.50-1.59(2H, m), 2.53(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.53(1H, td), 7.56(1H, d), 7.64(1H, td), 7.81(1H, d), 8.18(1H, dd), 8.50(1H, d)

Example B3

(4-Butylphenyl)(1-isoquinolyl)ketone



1-Bromo-4-butylbenzene (2.29 ml, 13 mmol) and a catalytic amount of 1,2-dibromoethane as an initiator were added to a mixed solution of magnesium (338 mg, 14 mmol) and tetrahydrofuran (6.5 ml) under nitrogen atmosphere, and this mixture was stirred under reflux for 10 minutes. The mixture was cooled to 0°C, a solution of 1-isoquinolinecarbonitrile (1.0 g, 6.5 mmol) in tetrahydrofuran was added, and this mixture was stirred at room temperature for 1 hour, then at 70°C for 3 hours. Thereafter, the mixture was cooled again to 0°C, concentrated hydrochloric acid (2.6 ml) and methanol (11 ml) were added, and this mixture was heated under reflux for 2 hours. After the mixture was concentrated, the residue was dissolved in 5 N sodium hydroxide and toluene, and was filtered through celite. The toluene layer of the filtrate was separated, washed with water, dried over anhydrous

magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (1.7 g).

^{1}H-NMR($CDCl_3$) δ (ppm):0.93(3H, t), 1.32-1.43(2H, m), 1.58-1.66(2H, m), 2.68(2H, t), 7.28(2H, d), 7.61(1H, td), 7.74(1H, td), 7.80(1H, d), 7.87(2H, d), 7.92(1H, d), 8.20(1H, d), 8.60(1H, d)

Example B4

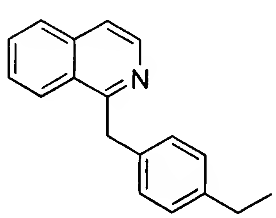
Alternative method for the production of 1-(4-butylbenzyl)isoquinoline

The compound of Example B3 (1.7 g, 6.0 mmol), hydrazine monohydrate (836 mg, 17 mmol), and potassium hydroxide (769 mg, 14 mmol) were added to diethylene glycol (8.5 ml), and this mixture was stirred at 80°C for 1 hour, at 160°C for 3.5 hours, then at 200°C for 1 hour. The mixture was cooled to room temperature, ice water was added, and this was extracted with ethyl acetate. The extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (914 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.50-1.59(2H, m), 2.53(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.53(1H, td), 7.56(1H, d), 7.64(1H, td), 7.81(1H, d), 8.18(1H, dd), 8.50(1H, d)

Example B5

1-(4-Ethylbenzyl)isoquinoline



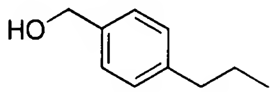
Using *p*-ethylbenzyl chloride, the title compound was obtained in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):1.18(3H, t), 2.57(2H, q), 4.64(2H, s), 7.08(2H, d), 7.20(2H, d), 7.50-7.55(2H, m), 7.61-7.65(1H, m), 7.80(1H, d),

8.16-8.18(1H, m), 8.49(1H, d)

Example B6

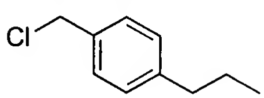
(4-Propylphenyl)methanol



A solution of sodium borohydride (2.9 g, 76 mmol) and concentrated sulfuric acid in ether (prepared by adding 2.0 ml of concentrated sulfuric acid to 4.0 ml of ether) was added dropwise to a solution of *p*-*n*-propylbenzoic acid (5.0 g, 32 mmol) in tetrahydrofuran (20 ml) cooled to 0°C keeping the temperature of the reaction system below 20°C, and then this mixture was stirred at room temperature for 3 hours. After the mixture was cooled on ice, methanol and 1 N sodium hydroxide were added, and this mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure to give the title compound (4.33 g). This compound was used in the following reaction without purification.

Example B7

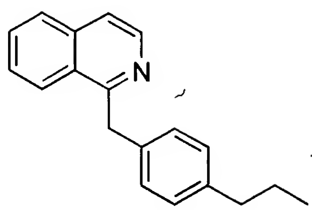
1-(Chloromethyl)-4-propylbenzene



The title compound was obtained by treating the compound of Example B6 in the same manner as in Example B1. This compound was used in the following reaction without further purification.

Example B8

1-(4-Propylbenzyl)isoquinoline

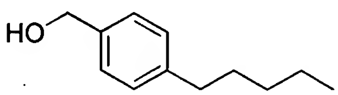


The title compound was obtained by treating the compound of Example B7 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.55-1.61(2H, m), 2.51(2H, t), 4.64(2H, s), 7.06(2H, d), 7.19(2H, d), 7.51-7.55(2H, m), 7.61-7.65(1H, m), 7.81(1H, d), 8.17(1H, dd), 8.49(1H, d)

Example B9

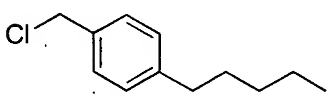
(4-Pentylphenyl)methanol



The title compound was obtained by reducing 4-*n*-amylbenzoic acid in the same manner as in Example B6.

Example B10

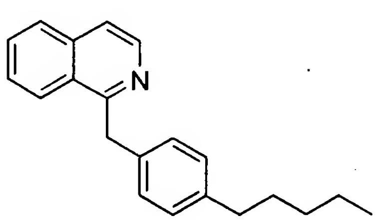
1-(Chloromethyl)-4-pentylbenzene



The title compound was obtained by treating the compound of Example B9 in the same manner as in Example B1. This compound was used in the following reaction without further purification.

Example B11

1-(4-Pentylbenzyl)isoquinoline

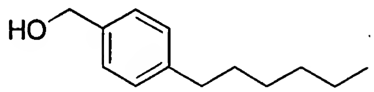


The title compound was obtained by treating the compound of Example B10 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.86(3H, t), 1.26-1.33(4H, m), 1.52-1.59(2H, m), 2.52(2H, t), 4.64(2H, s), 7.06(2H, d), 7.18(2H, d), 7.50-7.55(2H, m), 7.61-7.65(1H, m), 7.80(1H, d), 8.17(1H, dd), 8.49(1H, d)

Example B12

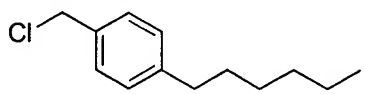
(4-Hexylphenyl)methanol



The title compound was obtained by reducing 4-*n*-hexylbenzoic acid in the same manner as in Example B6. This compound was used in the following reaction without further purification.

Example B13

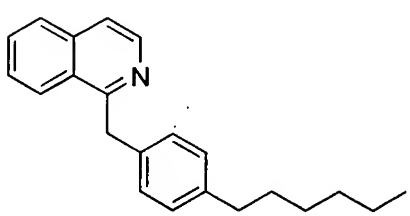
1-(Chloromethyl)-4-hexylbenzene



The title compound was obtained by treating the compound of Example B12 in the same manner as in Example B1. This compound was used in the following reaction without further purification.

Example B14

1-(4-Hexylbenzyl)isoquinoline



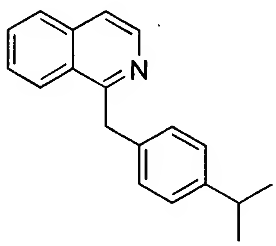
The title compound was obtained by treating the compound of Example B13 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.86(3H, t), 1.26-1.31(6H, m), 1.51-1.58(2H, m), 2.52(2H, t), 4.63(2H, s), 7.06(2H, d), 7.18(2H, d), 7.50-7.55(2H, m),

7.61-7.65(1H, m), 7.80(1H, d), 8.17(1H, dd), 8.49(1H, d)

Example B15

1-(4-Isopropylbenzyl)isoquinoline

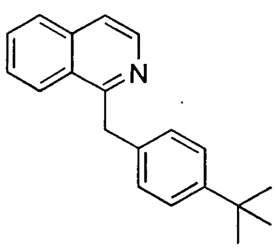


The title compound was obtained by treating *p*-isopropylbenzyl chloride in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):1.19(6H, d), 2.80-2.87(1H, m), 4.64(2H, s), 7.11(2H, d), 7.21(2H, d), 7.51-7.56(2H, m), 7.61-7.65(1H, m), 7.81(1H, d), 8.19(1H, dd), 8.50(1H, d)

Example B16

1-[4-(*tert*-Butyl)benzyl]isoquinoline

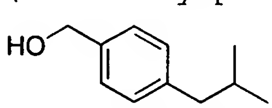


The title compound was obtained by treating 4-*tert*-butylbenzyl chloride in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):1.26(9H, s), 4.64(2H, s), 7.22(2H, d), 7.27(2H, d), 7.52-7.56(2H, m), 7.62-7.66(1H, m), 7.81(1H, d), 8.19(1H, dd), 8.50(1H, d)

Example B17

(4-Isobutylphenyl)methanol

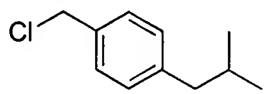


The title compound was obtained by reducing 4-isobutylbenzoic acid

in the same manner as in Example B6. This was used in the following reaction without further purification.

Example B18

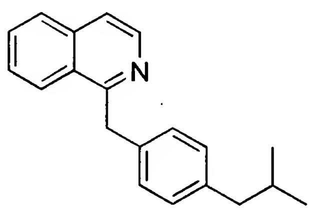
1-(Chloromethyl)-4-isobutylbenzene



The title compound was obtained by treating the compound of Example B17 in the same manner as in Example B1. This was used in the following reaction without further purification.

Example B19

1-(4-Isobutylbenzyl)isoquinoline

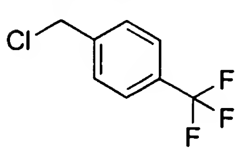


The title compound was obtained by treating the compound of Example B18 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.86(6H, d), 1.75-1.83(1H, m), 2.39(2H, d), 4.66(2H, s), 7.02(2H, d), 7.18(2H, d), 7.52-7.58(2H, m), 7.63-7.67(1H, m), 7.82(1H, d), 8.18(1H, d), 8.50(1H, d)

Example B20

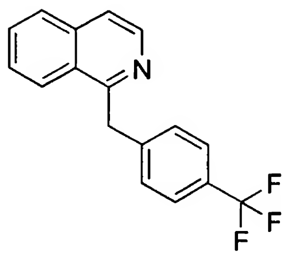
1-(Chloromethyl)-4-(trifluoromethyl)benzene



The title compound was obtained by treating 4-trifluoromethylbenzyl alcohol in the same manner as in Example B1. This was used in the following reaction without further purification.

Example B21

1-[4-(Trifluoromethyl)benzyl]isoquinoline

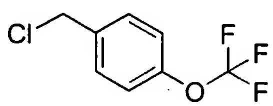


The title compound was obtained by treating the compound of Example B20 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.73(2H, s), 7.39(2H, d), 7.51(2H, d), 7.54-7.60(2H, m), 7.65-7.69(1H, m), 7.84(1H, d), 8.09-8.10(1H, m), 8.51(1H, d)

Example B22

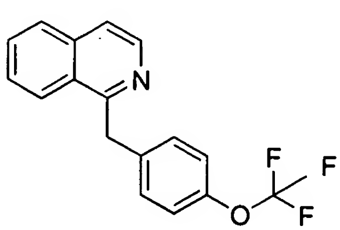
1-(Chloromethyl)-4-(trifluoromethoxy)benzene



The title compound was obtained by treating 4-trifluoromethoxybenzyl alcohol in the same manner as in Example B1. This was used in the following reaction without further purification.

Example B23

1-[4-(Trifluoromethoxy)benzyl]isoquinoline



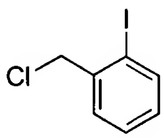
The title compound was obtained by treating the compound of Example B22 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.67(2H, s), 7.10(2H, d), 7.27(2H, d),

7.54-7.59(2H, m), 7.64-7.68(1H, m), 7.84(1H, d), 8.11(1H, dd), 8.50(1H, d)

Example B24

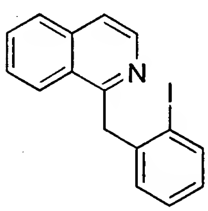
1-(Chloromethyl)-2-iodobenzene



Methanesulfonyl chloride (2.0 ml, 29 mmol) and triethylamine (3.6 ml, 26 mmol) were added to a solution of *o*-iodobenzyl alcohol (5.0 g, 21 mmol) in methylene chloride (50 ml) cooled to 0°C, and the mixture was stirred at that temperature for 19 hours. A 5% aqueous sodium hydrogencarbonate solution was added, and the resulting mixture was extracted with methylene chloride. The methylene chloride layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give the title compound (5.34 g).

Example B25

1-(2-Iodobenzyl)isoquinoline

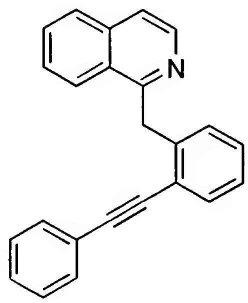


The title compound was obtained by treating the compound of Example B24 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.74(2H, s), 6.81-6.84(1H, m), 6.87-6.92(1H, m), 7.11-7.15(1H, m), 7.55-7.57(1H, m), 7.60(1H, d), 7.64-7.68(1H, m), 7.83-7.86(1H, m), 7.89-7.91(1H, m), 8.00-8.02(1H, m), 8.50(1H, d)

Example B26

1-[2-(2-Phenyl-1-ethynyl)benzyl]isoquinoline

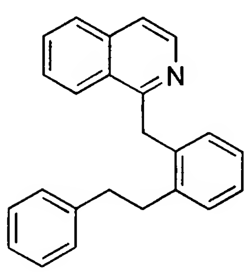


A solution of tetrakis(triphenylphosphine)palladium (58 mg, 0.05 mmol) and ethynylbenzene (204 mg, 2.0 mmol) in pyrrolidine (1.5 ml) was added to a solution of the compound of Example B25 (345 mg, 1.07 mmol) in pyrrolidine (1.5 ml) under nitrogen atmosphere, and the mixture was stirred at 80°C for 3 hours. The mixture was cooled to room temperature, diluted with ethyl acetate, washed with a saturated aqueous ammonium chloride solution, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography to give the title compound (280 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 4.95(2H, s), 6.98-7.06(2H, m), 7.10-7.21(2H, m), 7.31-7.35(3H, m), 7.48-7.51(3H, m), 7.57-7.65(2H, m), 7.82(1H, d), 8.25(1H, d), 8.52(1H, d)

Example B27

1-(2-Phenylethylbenzyl)isoquinoline



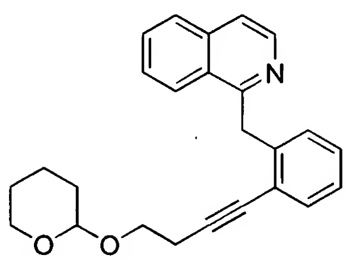
Palladium-carbon (10%, 230 mg) was added to a solution of the compound of Example B26 (280 mg, 0.88 mmol) in tetrahydrofuran (30 ml), and this mixture was stirred at room temperature under hydrogen atmosphere (1 atm) for 3 hours. The catalyst was removed by filtration and the obtained filtrate was concentrated under reduced pressure. The

residue was purified by silica gel chromatography to give the title compound (162 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 2.90-2.94 (2H, m), 3.07-3.10 (2H, m), 4.67 (2H, s), 6.80 (1H, d), 7.02-7.06 (1H, m), 7.15-7.30 (7H, m), 7.49-7.53 (1H, m), 7.58 (1H, d), 7.64-7.68 (1H, m), 7.84 (1H, d), 7.95 (1H, d), 8.50 (1H, d)

Example B28

1-{2-[4-(Tetrahydro-2*H*-2-pyranyloxy)-1-butynyl]benzyl}-isoquinoline

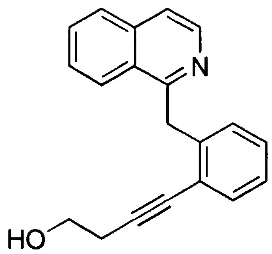


A solution of tetrakis(triphenylphosphine)palladium (58 mg, 0.05 mmol) and 2-(3-butynyloxy)-tetrahydro-2*H*-pyran (208 mg, 2.0 mmol) in pyrrolidine (1.5 ml) was added to a solution of the compound of Example B25 (345 mg, 1.07 mmol) in pyrrolidine (1.5 ml) under nitrogen atmosphere, and this mixture was stirred for four days at room temperature, and for another 30 minutes at 80°C. The mixture was cooled to room temperature, diluted with ethyl acetate, washed with a saturated aqueous ammonium chloride solution, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography to give the title compound (277 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 1.42-1.60 (4H, m), 1.64-1.68 (1H, m), 1.75-1.81 (1H, m), 2.76-2.80 (2H, m), 3.46-3.51 (1H, m), 3.60-3.66 (1H, m), 3.85-3.95 (2H, m), 4.64-4.66 (1H, m), 4.85 (2H, s), 6.95-6.98 (1H, m), 7.05-7.13 (2H, m), 7.44-7.46 (1H, m), 7.49-7.53 (1H, m), 7.56 (1H, d), 7.60-7.65 (1H, m), 7.80-7.82 (1H, m), 8.15-8.18 (1H, m), 8.49-8.51 (1H, m)

Example B29

4-[2-(1-Isoquinolylmethyl)phenyl]-3-butyn-1-ol

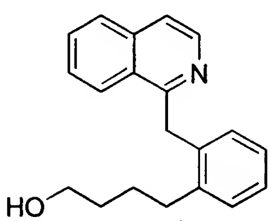


After the compound of Example B28 (200 mg, 0.54 mmol) was cooled to 0°C, a hydrochloric acid-methanol solution (10%, 5 ml) was added, and this mixture was stirred for 15 minutes. A saturated aqueous sodium hydrogencarbonate solution was added, and this mixture was extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give the title compound (86 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):2.72(2H, t), 3.53-3.60(1H, brs), 3.85(2H, t), 4.85(2H, s), 7.12-7.15(2H, m), 7.22-7.24(1H, m), 7.42-7.44(1H, m), 7.55-7.59(2H, m), 7.63-7.67(1H, m), 7.81(1H, d), 8.30(1H, m), 8.46(1H, m)

Example B30

4-[2-(1-Isoquinolylmethyl)phenyl]-1-butanol

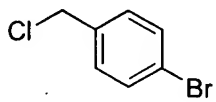


Palladium-carbon (10%, 10 mg) was added to a solution of the compound of Example B29 (44 mg, 0.15 mmol) in tetrahydrofuran (5 ml), and this mixture was stirred at room temperature under hydrogen atmosphere (1 atm) for 1 hour. After the catalyst was removed by filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography to give the title compound (18 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):1.61-1.75(4H, m), 2.33(1H, brs), 2.77(2H, t), 3.67(2H, t), 4.70(2H, s), 6.91(1H, d), 7.02-7.06(1H, m), 7.12-7.16(1H, m), 7.19-7.21(1H, m), 7.50-7.55(1H, m), 7.57(1H, d), 7.63-7.67(1H, d), 7.83(1H, d), 8.09(1H, d), 8.47(1H, d)

Example 31

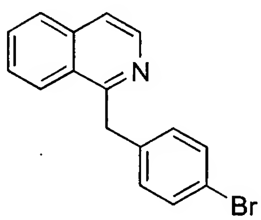
1-Bromo-2-(chloromethyl)benzene



The title compound was obtained by treating *p*-bromobenzyl alcohol in the same manner as in Example B1.

Example B32

1-(4-Bromobenzyl)isoquinoline

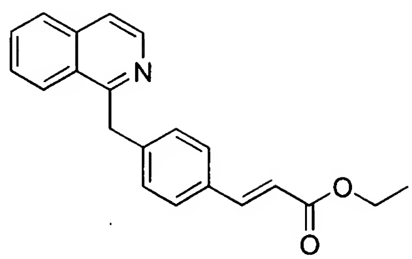


The title compound was obtained by treating the compound of Example B31 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.61(2H, s), 7.14-7.16(2H, m), 7.35-7.39(2H, m), 7.52-7.58(2H, m), 7.63-7.67(1H, m), 7.82(1H, d), 8.07-8.10(1H, m), 8.49(1H, d)

Example B33

Ethyl(*E*)-3-[4-(isoquinolylmethyl)phenyl]-2-propanoate

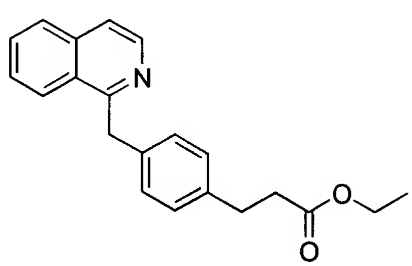


Tris(2-methylphenyl)phosphine (20 mg, 0.067 mmol), palladium(II) acetate (7.5 mg, 0.034 mmol), and triethylamine (70 μl, 0.50 mmol) were added to a solution of the compound of Example B32 (100 mg, 0.34 mmol) and vinyl propionate (73 μl, 0.67 mmol) in dimethylformamide (1.0 ml) under nitrogen atmosphere, and this mixture was stirred at 100°C for 4 hours. After the mixture was cooled to room temperature, water was added, and this mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (74 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):1.32(3H, t), 4.24(2H, q), 4.69(2H, s), 6.36(1H, d), 7.29(2H, d), 7.42(2H, d), 7.53-7.67(4H, m), 7.83(1H, d), 8.11-8.13(1H, m), 8.50(1H, d)

Example B34

Ethyl 3-[4-(1-isoquinolylmethyl)phenyl]propanoate



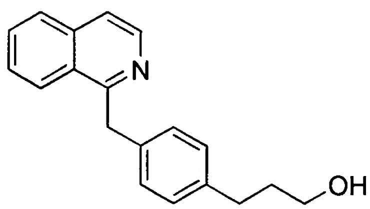
Palladium-carbon (10%, 20 mg) was added to a solution of the compound of Example B33 (71 mg, 0.22 mmol) in methanol (5.0 ml), and this reaction mixture was stirred at room temperature under hydrogen atmosphere at atmospheric pressure for 5 hours. After the catalyst was removed from the reaction mixture by filtration, the filtrate was

concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (52 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):1.20(3H, t), 2.56(2H, t), 2.88(2H, t), 4.09(2H, q), 4.64(2H, s), 7.09(2H, d), 7.20(2H, d), 7.51-7.57(2H, m), 7.62-7.66(1H, m), 7.82(1H, d), 8.15(1H, dd), 8.50(1H, d)

Example B35

3-[4-(1-Isoquinolylmethyl)phenyl]-1-propanol

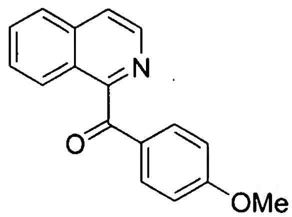


Lithium aluminum hydride (6 mg, 0.16 mmol) was added to tetrahydrofuran (1.0 ml) cooled to 0°C under nitrogen atmosphere. A solution of the compound of Example B34 (46 mg, 0.14 mmol) in tetrahydrofuran (1.0 ml) was further added, and this reaction mixture was stirred at that temperature for 3 hours. A mixed solution of methanol and water (9:1, 1.0 ml) was added to the reaction mixture, a saturated aqueous ammonium chloride solution was further added, then this mixture was extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (22 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):1.30-1.35(1H, brs), 1.81-1.88(2H, m), 2.64(2H, t), 3.62-3.65(2H, m), 4.64(2H, s), 7.09(2H, d), 7.20(2H, d), 7.51-7.57(2H, m), 7.62-7.66(1H, m), 7.81(1H, d), 8.16-8.18(1H, m), 8.49(1H, d)

Example 36

1-Isoquinolyl(4-methoxyphenyl)ketone

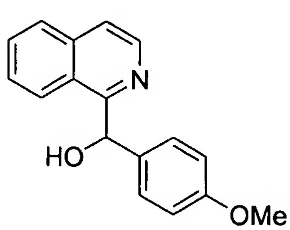


4-Bromoanisol (15.3 ml, 122 mmol) and a catalytic amount of 1,2-dibromoethane as an initiator were added to a mixed solution of magnesium (3059 mg, 125.8 mmol) and tetrahydrofuran (20 ml) under nitrogen atmosphere, and this reaction mixture was stirred while heating under reflux for 45 minutes. The mixture was cooled to 0°C, a solution of 1-isoquinolinecarbonitrile (10.78 g, 69.9 mmol) in tetrahydrofuran (30 ml) was added dropwise thereto, and this reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was cooled on ice, concentrated hydrochloric acid (24 ml) and methanol (120 ml) were added, and this mixture was heated under reflux for 1.5 hours. After cooling on ice, the mixture was adjusted to pH 8 by adding aqueous sodium hydroxide, extracted with ether, washed with saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (15.87 g).

^{1}H-NMR($CDCl_3$) δ (ppm):3.88(3H, s), 6.95(2H, d), 7.61(1H, dd), 7.74(1H, dd), 7.76(1H, d), 7.85(2H, d), 8.17(1H, dd), 8.60(1H, d).

Example B37

1-Isoquinolyl(4-methoxyphenyl)methanol



Sodium borohydride (1855 mg) was added to an ice-cooled solution of the compound of Example B36 (8608 mg) in ethanol (170 ml), and this mixture was stirred at room temperature for 35 minutes. Sodium

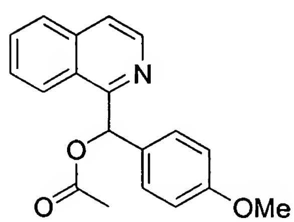
borohydride (957 mg) was further added, and this reaction mixture was stirred at 40°C for 40 minutes. The reaction mixture was concentrated under reduced pressure, water was added, and this mixture was extracted with ether. The organic layer was washed with water and saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The obtained title compound (7881 mg) was used in the following reaction without further purification.

^{1}H-NMR(DMSO-d6) δ (ppm):3.66(3H, s), 6.30-6.32(1H, brs), 6.81(2H, d), 7.28(2H, d), 7.54(1H, dd), 7.68(1H, dd), 7.76(1H, d), 7.94(1H, d), 8.37(1H, d), 8.47(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B38

1-Isoquinolyl(4-methoxyphenyl)methyl acetate

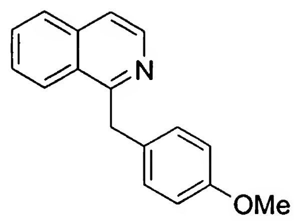


Acetic anhydride (20 ml) was added to a solution of the compound of Example B37 (7881 mg) in pyridine (100 ml), and this reaction mixture was stirred at 50°C for 4 hours. The reaction mixture was concentrated under reduced pressure and subjected to azeotropic distillation with toluene. The residue was purified by silica gel column chromatography to give the title compound (8.79 g).

^{1}H-NMR($CDCl_3$) δ (ppm):2.22(3H, s), 3.76(3H, s), 6.84(2H, d), 7.39(2H, d), 7.54(1H, dd), 7.56(1H, s), 7.60(1H, d), 7.64(1H, dd), 7,82(1H, d), 8.19(1H, d), 8.57(1H, d).

Example B39

1-(4-Methoxybenzyl)isoquinoline

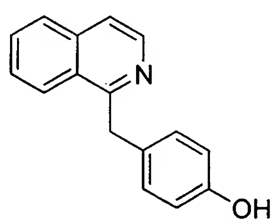


Palladium-carbon (10%, 4.0 g) was added to a solution of the compound of Example B38 (8.79 g) in methanol (150 ml), and this mixture was stirred at room temperature under hydrogen atmosphere at atmospheric pressure for 5.5 hours. The catalyst was removed by filtration through celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (4.48 g).

^{1}H-NMR($CDCl_3$) δ (ppm):3.74(3H, s), 4.61(2H, s), 6.79(2H, d), 7.21(2H, d), 7.53(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.16(1H, d), 8.49(1H, d).

Example B40

4-(1-Isoquinolylmethyl)phenol



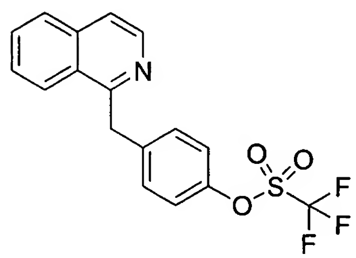
An aqueous hydrobromic acid solution (47%, 40 ml) was added to the compound of Example B39 (2185 mg), and this reaction mixture was heated under reflux for 14 hours. The reaction mixture was cooled to room temperature, further cooled on ice, neutralized with a 50% aqueous sodium hydroxide solution, and extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The obtained powder was washed with petroleum ether to give the title compound (1822 mg).

^{1}H-NMR(DMSO-d6) δ (ppm):4.48(2H, s), 6.61(2H, d), 7.07(2H, d), 7.60(1H,

dd), 7.68(1H, d), 7.71(1H, dd), 7.92(1H, d), 8.27(1H, d), 8.41(1H, d), 9.19(1H, brs).

Example B41

4-(1-Isoquinolylmethyl)phenyl trifluoromethanesulfonate

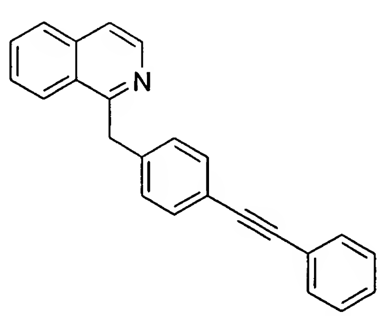


Trifluoromethanesulfonic anhydride (0.55 ml) was added dropwise to an ice-cold solution of the compound of Example B40 (513 mg) in pyridine (10 ml), and this reaction mixture was stirred at that temperature for 45 minutes. After ice was added, the reaction mixture was extracted with ether. The organic layer was washed with water and saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (546 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):4.69(2H, s), 7.16(2H, d), 7.35(2H, d), 7.57(1H, dd), 7,60(1H, d), 7.68(1H, dd), 7.85(1H, d), 8.09(1H, d), 8.50(1H, d).

Example B42

1-[4-(2-Phenyl-1-ethynyl)benzyl]isoquinoline



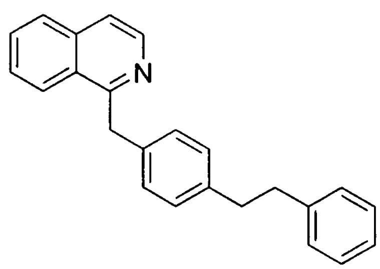
Phenylacetylene (53 μl), palladium acetate (9 mg), 1,1'-bis(diphenylphosphino)ferrocene (67 mg), copper(I) iodide (3 mg),

lithium chloride (20 mg), and triethylamine (50 µl) were added to a solution of the compound of Example B41 (88 mg) in *N*, *N*-dimethylformamide (2.0 ml) that had been degassed and placed under nitrogen, and this mixture was stirred at 80°C for 8 hours. After cooling the mixture to room temperature, water was added, and this mixture was extracted with ethyl acetate. The organic layer was washed with water and saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (53 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):4.69(2H, s), 7.12-7.32(3H, m), 7.25(2H, d), 7.42(2H, d),7.43-7.52(2H, m), 7.54(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.10(1H, d), 8.51(1H, d).

Example B43

1-(4-Phenethylbenzyl)isoquinoline

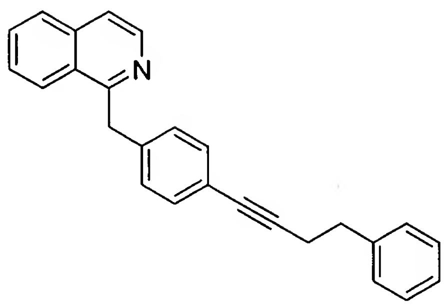


Palladium-carbon catalyst (10%, 20 mg) was added to a solution of the compound of Example B42 (45 mg) in tetrahydrofuran (2 ml), and this mixture was stirred at room temperature under hydrogen atmosphere at atmospheric pressure for 2 hours. The catalyst was removed by filtration through celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (23 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):2.78-2.90(4H, m), 4.64(2H, s), 7.07(2H, d), 7.10-7.20(5H, m), 7.22(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.15(1H, d), 8.49(1H, d).

Example B44

1-[4-(4-Phenyl-1-butynyl)benzyl]isoquinoline

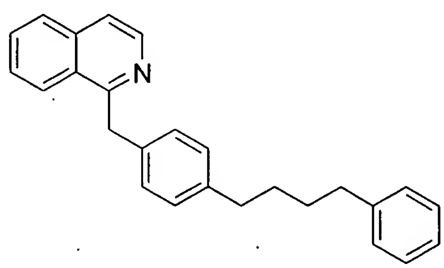


The title compound was obtained by treating the compound of Example B41 and 4-phenyl-1-butyne in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):2.65(2H, t), 2.88(2H, t), 4.68(2H, s), 7.12-7.40(9H, m), 7.50-7.70(3H, m), 7.80-7.88(1H, m), 8.00-8.10(1H, m), 8.48-8.51(1H, m).

Example B45

1-[4-(4-Phenyl-1-butyl)benzyl]isoquinoline

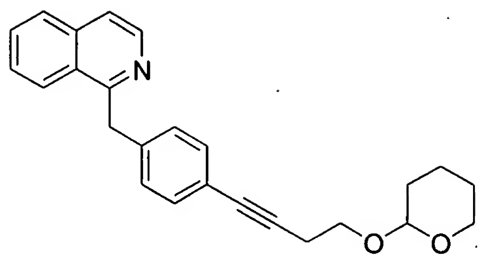


The title compound was obtained by treating the compound of Example B44 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.55-1.80(4H, m), 2.50-2.65(4H, m), 4.68(2H, s), 7.00-7.30(9H, m), 7.52(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.81(1H, d), 8.15(1H, d), 8.50(1H, d).

Example 46

1-{4-[4-(tetrahydro-2*H*-2-pyranyloxy)-1-butynyl]benzyl}-isoquinoline

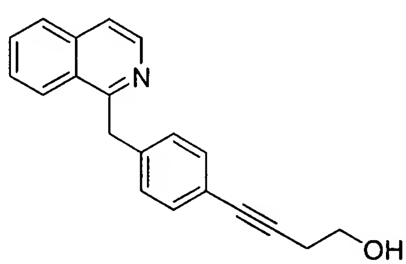


The title compound was obtained by treating the compound of Example B41 and 2-(3-butynyloxy)tetrahydro-2*H*-pyran in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.48-1.90(6H, m), 2.67(2H, t), 3.49-3.55(1H, m), 3.60(1H, dd), 3.65-3.94(2H, m), 4.66(2H, s), 4.65-4.70(1H, m), 7.14-7.20(2H, m), 7.23-7.30(2H, m), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.10(1H, d), 8.49(1H, d).

Example B47

4-[4-(1-Isoquinolylmethyl)phenyl]-3-butyn-1-ol



The compound of Example B46 (1048 mg) was dissolved in a 10% hydrochloric acid-methanol solution (50 ml), and this reaction mixture was stirred at room temperature for 1.5 hours. The reaction mixture was cooled on ice, a saturated aqueous sodium hydrogencarbonate solution was added, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with water and saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (666 mg).

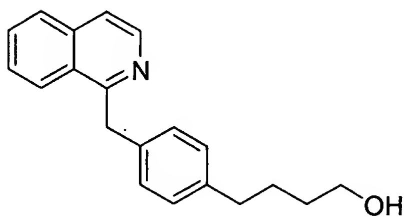
^{1}H-NMR($CDCl_3$) δ (ppm):2.65(2H, t), 3.77(2H, t), 4.65(2H, s), 7.18(2H, d), 7.29(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d),

8.07(1H, d), 8.49(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B48

4-[4-(1-Isoquinolylmethyl)phenyl]-1-butanol



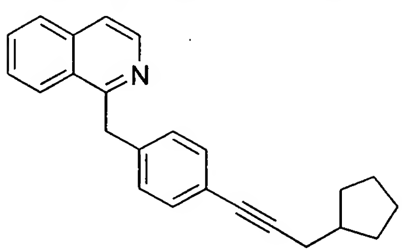
The title compound was obtained by treating the compound of Example B47 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.50-1.70(4H, m), 2.57(2H, t), 3.62(2H, t), 4.64(2H, s), 7.06(2H, d), 7.18(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.16(1H, d), 8.49(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example 49

1-[4-(3-Cyclopentyl-1-propynyl)benzyl]isoquinoline

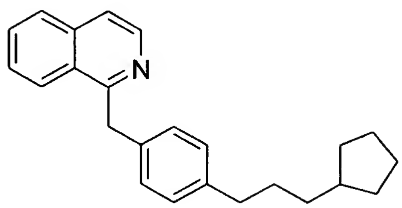


The title compound was obtained by treating the compound of Example B41 and 3-cyclopentyl-1-propyne in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.25-1.35(2H, m), 1.45-1.70(6H, m), 1.75-1.85 (2H, m), 2.05-2.13(1H, m), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.51(1H, dd), 7.56(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.49(1H, d).

Example B50

1-[4-(3-Cyclopentylpropyl)benzyl]isoquinoline

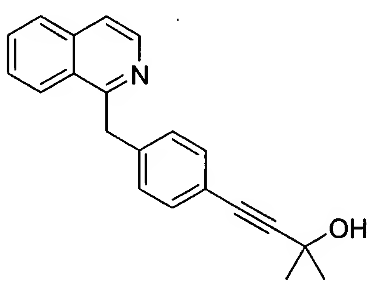


The title compound was obtained by treating the compound of Example B49 in the same manner as in Example B43.

[1]H-NMR($CDCl_3$) δ (ppm):1.25-1.74(13H, m), 2.49-2.54(2H, m), 4.64(2H, s), 7.06(2H, d), 7.18(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.17(1H, d), 8.49(1H, d).

Example B51

4-[4-(1-Isoquinolylmethyl)phenyl]-2-methyl-3-butyn-2-ol

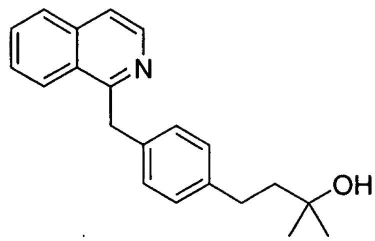


The title compound was obtained by treating the compound of Example B41 and 2-methyl-3-butyn-2-ol in the same manner as in Example B42.

[1]H-NMR(DMSO-d6) δ (ppm):1.35(1H, s), 1.40(6H, s), 4.62(2H, s), 7.20-7.30(4H, m), 7.61(1H, dd), 7.71(1H, d), 7.69-7.76(1H, m), 7.95(1H, d), 8.26(1H, d), 8.42(1H, d).

Example B52

4-[4-(1-Isoquinolylmethyl)phenyl]-2-methyl-2-butanol



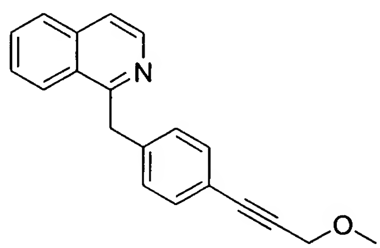
The title compound was obtained by treating the compound of Example B51 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.25(6H, s), 1.70-1.77(2H, m), 2.60-2.67(2H, m), 4.64(2H, s), 7.08(2H, d), 7.19(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.16(1H, d), 8.49(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B53

1-[4-(3-Methoxy-1-propynyl)benzyl]isoquinoline

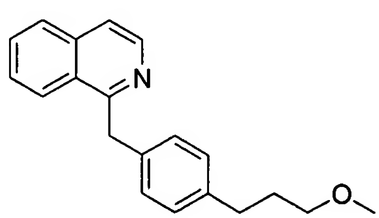


The title compound was obtained by treating the compound of Example B41 and methylpropargyl ether in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):3.42(3H, s), 4.29(2H, s), 4.66(2H, s), 7.21 (2H, d), 7.34(2H, d), 7.54(1H, dd), 7.58(1H, d), 7.65(1H, dd) 7.82(1H, d), 8.10(1H, d) 8.49(1H, d).

Example B54

1-[4-(3-Methoxypropyl)benzyl]isoquinoline

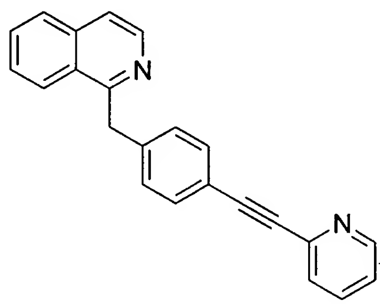


The title compound was obtained by treating the compound of Example B53 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.78-1.87 (2H, m), 2.06(2H, t), 3.31(3H, s), 3.35(2H, t), 4.64(2H, s), 7.07(2H, d), 7.22(2H, d), 7.53(1H, dd), 7.55(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.17(1H, d),8.49(1H, d).

Example B55

1-{4-[2-(2-Pyridyl)-1-ethynyl]benzyl}isoquinoline

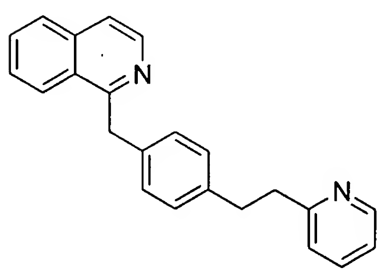


The title compound was obtained by treating the compound of Example B41 and 2-ethynylpyridine in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):4.71(2H, s), 7.20-7.25(2H, m), 7.29(2H, d), 7.48-7.53(1H, m), 7.51(2H, d), 7.57(1H, dd), 7.61(1H, d), 7.67(1H, dd), 7.85(1H, d), 8.13(1H, d), 8.53(1H, d), 8.59-8.63(1H, m).

Example B56

1-{4-[2-(2-Pyridyl)ethyl]benzyl}isoquinoline



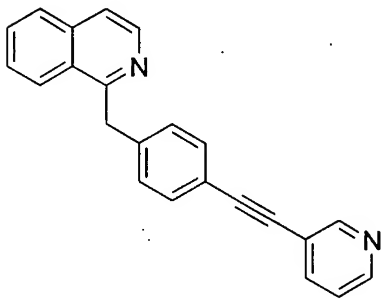
The title compound was obtained by treating the compound of Example B55 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):2.94-3.06(4H, m), 4.64(2H, s), 7.04(1H, d), 7.09(1H, dd), 7.09(2H, d), 7.18(2H, d), 7.53(1H, ddd), 7.54(1H, dd),

7.55(1H, d), 7.64(1H, d), 7.81(1H, d), 8.15(1H, d), 8.49(1H, d), 8.53(1H, dd).

Example B57

1-{4-[2-(3-pyridyl)-1-ethynyl]benzyl}isoquinoline

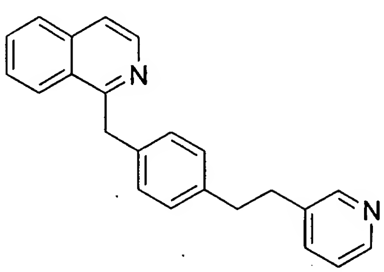


The title compound was obtained by treating the compound of Example B41 and 3-ethynylpyridine in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):4.69(2H, s), 7.27(2H, d), 7.31(1H, dd), 7.43(2H, d), 7.55(1H, dd), 7.59(1H, d), 7.66(1H, dd), 7.82(1H, ddd), 7.83(1H, d), 8.10(1H, d), 8.51(1H, d), 8.60(1H, dd), 8.77(1H, d).

Example B58

1-{4-[2-(3-Pyridyl)ethyl]benzyl}isoquinoline

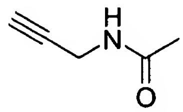


The title compound was obtained by treating the compound of Example B57 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):2.80-2.90(4H, m), 4.65(2H, s),7.04(2H, d), 7.15(1H, dd), 7.19(2H, d), 7.39(1H, dd), 7.54(1H, dd), 7.56(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.15(1H, d), 8.40(1H, d), 8.42(1H, d), 8.49(1H, d).

Example B59

N-(2-propynyl)acetamide

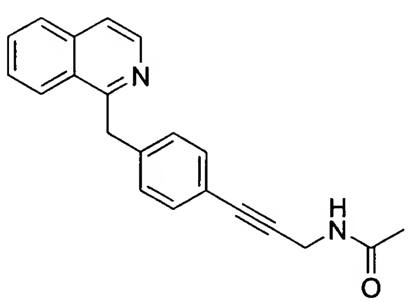


Pyridine (16.3 ml) and acetic anhydride (10.4 ml) were added to an ice-cooled solution of propargylamine (3023 mg) in methylene chloride (30 ml), and this reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was poured on ice, extracted with ethyl acetate, washed successively with 1 N hydrochloric acid, a saturated aqueous sodium hydrogencarbonate solution, and saturated brine, dried over anhydrous magnesium sulfate, and then filtered through silica gel. The filtrate was concentrated under reduced pressure to give the title compound (743 mg). The obtained compound was used in the following reaction without further purification.

^{1}H-NMR(DMSO-d6) δ (ppm):1.79(3H, s), 3.07(1H, t), 3.81(2H, d), 8.25(1H, brs).

Example B60

N-{3-[4-(1-Isoquinolylmethyl)phenyl]-2-propynyl}acetamide

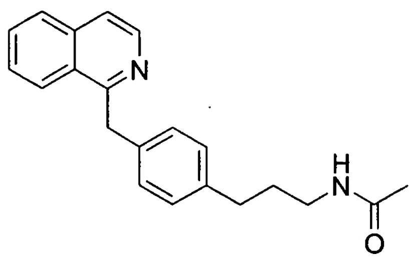


The title compound was obtained by treating the compound of Example B41 and the compound of Example B59 in the same manner as in Example B42.

^{1}H-NMR(DMSO-d6) δ (ppm):1.79(3H, s), 4.04(2H, s), 4.61(2H, s), 7.45-7.68(4H, m), 7.68-7.75(2H, m), 7.90-8.00(1H, m), 8.25-8.38(2H, m), 8.40-8.45(1H, m).

Example B61

N-{3-[4-(1-Isoquinolylmethyl)phenyl]propyl}acetamide

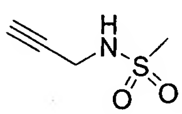


The title compound was obtained by treating the compound of Example B60 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.95(3H, s), 1.74-1.84(2H, m), 2.55(2H, t), 3.25(2H, dt), 4.68(2H, s), 7.10(2H, d), 7.18(2H, d), 7.20-7.28(1H, m), 7.50-7.58(2H, m), 7.60-7.68(1H, m), 7.75-7.85(1H, m), 8.10-8.16(1H, m), 8.45-8.50(1H, m).

Example B62

N-(2-Propynyl)methanesulfonamide



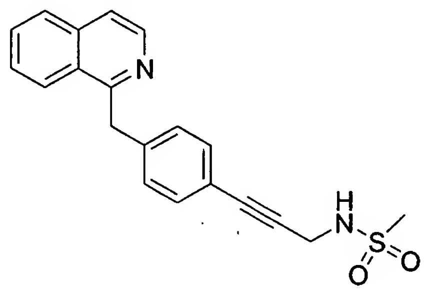
Triethylamine (9.77 ml) was added to an ice-cooled solution of propargylamine (3023 mg) in methylene chloride (30 ml). After dropwise addition of methanesulfonyl chloride (5.19 ml), the reaction mixture was stirred for 3 hours at that temperature, warmed to room temperature, and further stirred for 2 hours. Ice was added to the reaction mixture, the resulting mixture was extracted with ethyl acetate, washed with saturated brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in methanol (120 ml), potassium carbonate (11.7 g) was added, and this reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated under reduced pressure, neutralized with dilute hydrochloric acid while cooling on ice, and then extracted with ethyl acetate. The extract was washed with saturated brine, dried

over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (6.67 g).

^{1}H-NMR($CDCl_3$) δ (ppm):2.39(1H, t), 3.10(3H, s), 3.99(2H, dd), 4.60(1H, brs).

Example B63

N-{3-[4-(1-isoquinolylmethyl)phenyl]-2-propynyl}-methanesulfonamide

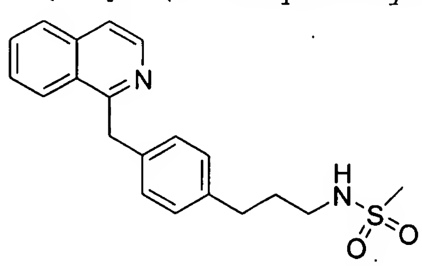


The title compound was obtained by treating the compound of Example B41 and the compound of Example B62 in the same manner as in Example B42.

^{1}H-NMR(DMSO-d6) δ (ppm):2.97(3H, s), 4.00(2H, d), 4.63(2H, s), 7.25-7.37(4H, m), 7.57(1H, t), 7.62(1H, dd), 7.71(1H, d), 7.73(1H, dd), 7.94(1H, d), 8.28(1H, d), 8.42(1H, d).

Example B64

N-{3-[4-(1-isoquinolylmethyl)phenyl]propyl}methanesulfonamide



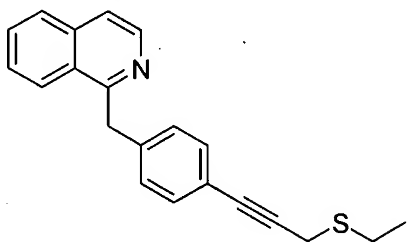
The title compound was obtained by treating the compound of Example B63 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.80-1.90(2H, m), 2.62(2H, t), 2.89(3H, s), 3.11(2H, dt), 4.25(1H, brs), 4.64(2H, s), 7.05(2H, d), 7.20(2H, d),

7.50(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.81(1H, d), 8.15(1H, d), 8.49(1H, d).

Example B65

1-{4-[3-(Ethylsulfanyl)-1-propynyl]benzyl}isoquinoline

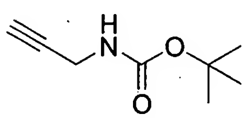


The title compound was obtained by treating the compound of Example B41 and propargyl ethyl sulfide in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.30(3H, t), 2.73(2H, q), 3.47(2H, s), 4.67(2H, s), 7.20-7.32(4H, m), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.49(1H, d).

Example B66

t-Butyl *N*-(propynyl)carbamate

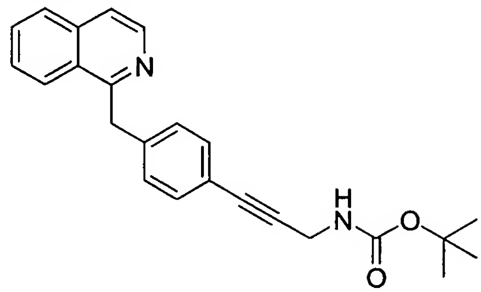


A solution of di-*t*-butyl-dicarbonate (10.84 g) in tetrahydrofuran (20 ml) was added dropwise to an ice-cooled solution of propargylamine (3040 mg) in tetrahydrofuran (20 ml), the temperature of the mixture was gradually raised to room temperature, and the reaction mixture was stirred for 20 hours. After water was added, the reaction mixture was extracted with ethyl acetate, washed with saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure to give the title compound (9.34 g). The obtained compound was used in the following reaction without further purification.

^{1}H-NMR(DMSO-d6) δ (ppm):1.36(9H, s),3.04(1H, t),3.62-3.70(2H, m),7.20-7.30(1H, m)

Example B67

tert-Butyl *N*-{3-[4-(1-isoquinolylmethyl)phenyl]-2-propynyl}-carbamate

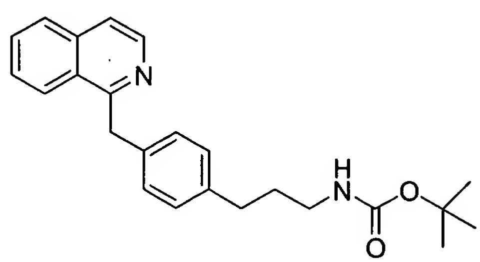


The title compound was obtained by treating the compound of Example B41 and the compound of Example B66 in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.45(9H, s), 4.06-4.13(2H, m), 4.66(2H, s), 7.19(2H, d), 7.20-7.28(1H, m), 7.29(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.08(1H, d), 8.49(1H, d).

Example B68

tert-Butyl *N*-{3-[4-(1-isoquinolylmethyl)phenyl]propyl}carbamate

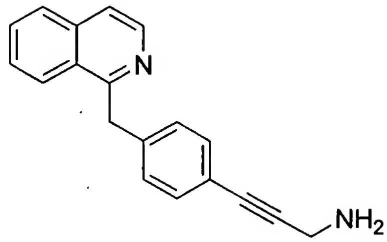


The title compound was obtained by treating the compound of Example B67 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.43(9H, s), 1.70-1.81(2H, m), 2.54-2.60(2H, m), 3.01-3.20(2H, m), 4.47-4.57(1H, m), 4.65(2H, s), 7.07(2H, d), 7.21(2H, d), 7.55(1H, dd), 7.57(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.18(1H, d), 8.51(1H, d).

Example B69

3-[4-(1-Isoquinolylmethyl)phenyl]-2-propyn-1-amine



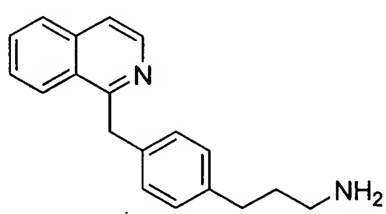
Trifluoroacetic acid (0.3 ml) was added to an ice-cooled solution of the compound of Example B67 (4 mg) in methylene chloride (0.6 ml), and the reaction mixture was stirred at that temperature for 1 hour. After a saturated aqueous sodium hydrogencarbonate solution was added, the reaction mixture was extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (4 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):3.60-3.68(2H, m),4.66(2H, s),7.19(2H, d), 7.29(2H, d), 7.53(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.82(1H, d), 8.10(1H, d), 8.49(1H, d).

The amine proton was not observed in the NMR spectrum.

Example B70

3-[4-(1-Isoquinolylmethyl)phenyl]-1-propanamine

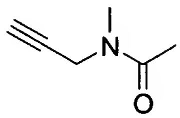


The title compound was obtained by treating the compound of Example B68 in the same manner as in Example B69.

^{1}H-NMR($CDCl_3$) δ (ppm):1.20-1.30(2H, m), 1.78-1.88(2H, m), 2.45-2.52(2H, m), 2.73-2.81(2H, m), 4.55(2H, s), 6.94(2H, d), 7.08(2H, d), 7.50(1H, dd), 7.51(1H, d), 7.61(1H, dd), 7.76(1H, d), 8.10(1H, d), 8.38(1H, d).

Example B71

N-methyl-*N*-(2-propynyl)acetamide



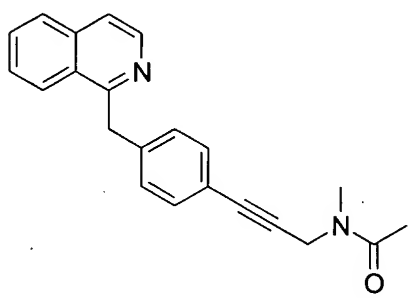
The title compound was obtained by treating *N*-methyl-*N*-(2-propynyl)amine in the same manner as in Example B59.

^{1}H-NMR($CDCl_3$) δ (ppm):2.11(2.1H, s), 2.17(0.9H, s),2.21(0.7H, t), 2.31(0.3H, t), 3.00(0.9H, s), 3.08(2.1H, s), 4.04(0.6H, d), 4.23(1.4H, d).

The obtained compound contained a 7:3 mixture of geometrical isomers of the amide.

Example B72

N-{3-[4-(1-isoquinolylmethyl)phenyl]-2-propynyl}-*N*-methyl-acetamaide



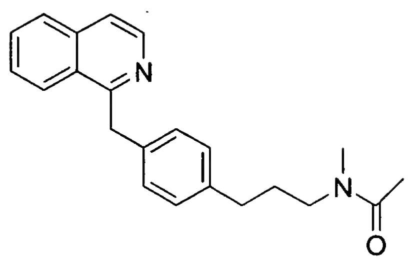
The title compound was obtained by treating the compound of Example B41 and the compound of Example B71 in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):2.10(1.8H, s), 2.11(1.2H, s), 3.01(1.2H, s), 3.10(1.8H, s), 4.21(1.2H, s), 4.41(0.8H, s), 4.67(2H, s), 7.18-7.23(2H, m), 7.29-7.32(2H, m), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

The obtained compound contained a 3:2 mixture of geometrical isomers of the amide.

Example B73

N-{3-[4-(1-isoquinolylmethyl)phenyl]propyl}-*N*1-methylacetamide



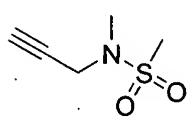
The title compound was obtained by treating the compound of Example B72 in the same manner as in Example B43.

^{1}H-NMR($CDCl_3$) δ (ppm):1.70-1.90(2H, m), 1.89(1.5H, s), 2.03(1.5H, s), 2.50-2.59(2H, m), 2.88(1.5H, s), 2.91(1.5H, s), 3.20-3.25(1H, m), 3.36-3.40(1H, m), 4.66(2H, s), 7.03-7.10(2H, m), 7.18-7.30(2H, m), 7.53(1H, dd), 7.58(1H, d), 7.66(1H, dd), 7.82(1H, d), 8.17(1H, d), 8.50(1H, d).

The obtained compounds contained a 1:1 mixture of geometrical isomers of the amide.

Example B74

N-methyl-*N*-(2-propynyl)methanesulfonamide

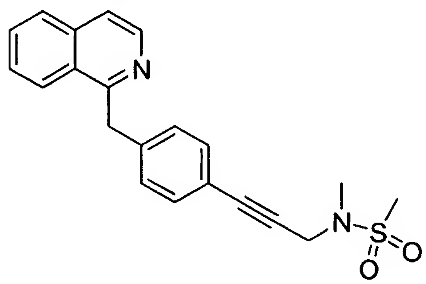


Triethylamine (6.55 ml) was added to an ice-cooled solution of *N*-methyl- *N*-(2-propynyl)amine (2603 mg) in methylene chloride (25 ml). Methanesulfonyl chloride (3.50 ml) was further added dropwise, the reaction mixture was stirred at that temperature for 1 hour, and then stirred further at room temperature for 2 hours. After ice was added, the reaction mixture was extracted with ethyl acetate, washed successively with 1 N hydrochloric acid, a saturated aqueous sodium hydrogencarbonate solution, and saturated brine, dried over anhydrous magnesium sulfate, and then filtered through silica gel. The filtrate was concentrated under reduced pressure to give the title compound (4522 mg). The obtained compound was used in the following reaction without further purification.

^{1}H-NMR($CDCl_3$) δ (ppm):2.41(1H, t), 2.93(3H, s), 2.96(3H, s), 4.09(2H, d).

Example B75

N-{3-[4-(1-isoquinolylmethyl)phenyl]-2-propynyl}-*N*-methyl methanesulfonamide

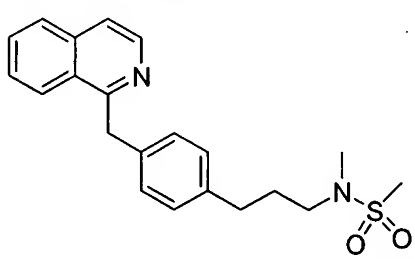


The title compound was obtained by treating the compound of Example B41 and the compound of Example B74 in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):2.95(3H, s), 2.97(3H, s), 4.26(2H, s), 4.68(2H, s), 7.24(2H, d), 7.31(2H, d), 7.55(1H, dd), 7.59(1H, d), 7.66(1H, dd), 7.83(1H, d), 8.10(1H, d), 8.49(1H, d).

Example B76

N-{3-[4-(1-isoquinolylmethyl)phenyl]propyl}-*N*-methyl methane-sulfonamide

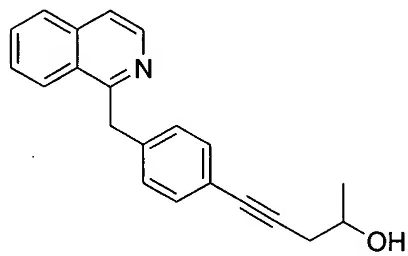


Treating the compound of Example B75 in the same manner as in Example B43, the obtained residue was separated and purified by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)] to give the title compound.

MS m/z(ESI:MH^+):369.2

Example B77

5-[4-(1-Isoquinolylmethyl)phenyl]-4-pentyn-2-ol



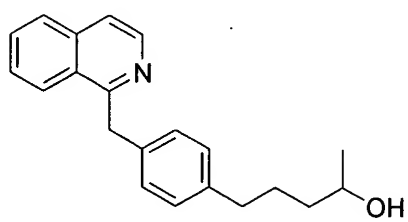
The title compound was obtained by treating the compound of Example B41 and 4-pentyn-2-ol in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.27(3H, t), 2.38-2.62(2H, m), 3.95-4.03(1H, m), 4.65(2H, s), 7.19(2H, d), 7.29(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.48(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B78

5-[4-(1-Isoquinolylmethyl)phenyl]-2-pentanol

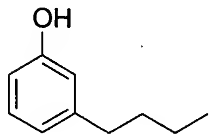


Treating the compound of Example B77 in the same manner as in Example B43, the obtained residue was separated and purified by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)] to give the title compound.

MS m/z(ESI:MH^+):306.2

Example B79

3-Butylphenol

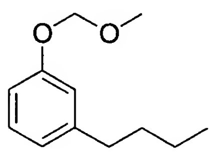


The title compound was obtained by treating 1-butyl-3-methoxybenzene in the same manner as in Example B40.

^{1}H-NMR($CDCl_3$) δ (ppm):0.94(3H, t), 1.30-1.55(2H, m), 1.55-1.62(2H, m), 2.56(2H, t), 4.76(1H, brs), 6.63(1H, dd), 6.66(1H, d), 6.75(1H, d), 7.12(1H, dd).

Example B80

1-Butyl-3-(methoxymethoxy)benzene

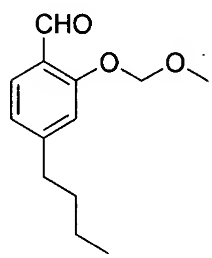


A 60% suspension of sodium hydride dispersed in mineral oil (102 mg) was added to an ice-cooled solution of the compound of Example B79 (318 mg) in dimethylformamide (5 ml), and the reaction mixture was stirred at room temperature for 30 minutes. The mixture was cooled again on ice, chloromethyl methyl ether (0.18 ml) was added, and this reaction mixture was stirred at room temperature for 12 hours. After water was added, the reaction mixture was extracted with ethyl acetate, washed with a saturated aqueous sodium hydrogencarbonate solution and saturated brine, dried over anhydrous magnesium sulfate, and then filtered through silica gel. The filtrate was concentrated under reduced pressure to give the title compound (341 mg). The obtained compound was used in the following reaction without further purification.

^{1}H-NMR($CDCl_3$) δ (ppm):0.94(3H, t), 1.30-1.42(2H, m), 1.55-2.04(2H, m), 2.58(2H, t), 3.49(3H, s), 5.17(2H, s), 6.80-6.87(3H, m), 7.18(1H, dd).

Example B81

4-Butyl-2-(methoxymethoxy)benzaldehyde

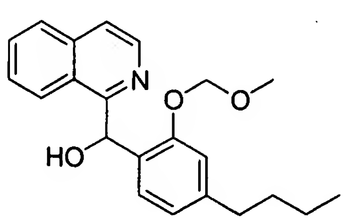


A solution of *t*-butyl lithium in pentane (1.51 M, 10.6 ml) was added dropwise to a solution of the compound of Example B80 (2396 mg) in petroleum ether cooled to -20°C, and this reaction mixture was stirred at a temperature in the range of -10°C to 0°C for 1.5 hours. The reaction mixture was cooled to -70°C, anhydrous ether (17 ml) and dimethylformamide (1.91 ml) were added, and the resulting mixture was stirred at that temperature for 3 hours, then stirred for another 1 hour at room temperature. The reaction mixture was cooled on ice, a saturated aqueous ammonium chloride solution was added, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (1821 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.94(3H, t), 1.32-1.42(2H, m), 1.57-1.65(2H, m), 2.64(2H, t), 3.54(3H, s), 5.29(2H, s), 6.91(1H, d), 7.01(1H, s),7.76(1H, d),10.44(1H, s).

Example B82

[4-Butyl-2-(methoxymethoxy)phenyl](1-isoquinolyl)methanol



An aqueous sodium hydroxide solution (50%, 1.4 ml) was added to

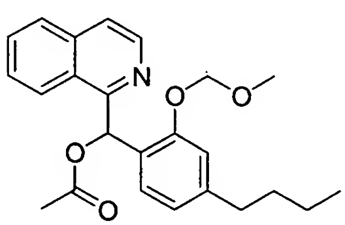
a solution of 1-cyano-benzoyl-1,2-dihydroisoquinoline (815 mg), which was synthesized according to Org. Synth., IV, 155 (1988), the compound of Example B81 (869 mg), and triethylbenzylammonium chloride (7 mg) in methylene chloride (1.6 ml) , and the reaction mixture was subjected to ultrasonication in a water bath for 10 minutes. After methylene chloride (8.3 ml) and ethanol (4.4 ml) were added, the reaction mixture was further subjected to ultrasonication in a water bath for 85 minutes. Water was added and the resulting reaction mixture was extracted with methylene chloride. The extract was dried over anhydrous magnesium sulfate, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (1144 mg).

^{1}H-NMR(DMSO-d6) δ (ppm):0.86(3H, t), 1.22-1.31(2H, m), 1.44-1.52(2H, m), 2.44-2.51(2H, m), 3.16(3H, s), 5.10(1H, d), 5.12(1H, d), 6.72(1H, s), 6.75(1H, d), 6.84(1H, s), 7.21(1H, d), 7.61(1H, dd), 7.72(1H, dd), 7.74(1H, d), 7.95(1H, d), 8.31(1H, d), 8.42(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B83

[4-Butyl-2-(methoxymethoxy)phenyl](1-isoquinolyl)methyl acetate

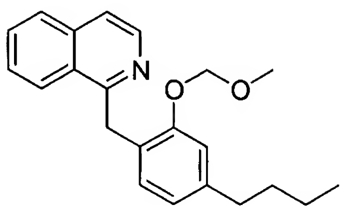


The title compound was obtained by treating the compound of Example B82 in the same manner as in Example B38.

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.28-1.40(2H, m), 1.50-1.60(2H, m), 2.22(3H, s), 2.54(2H, t), 3.41(3H, s), 5.22(1H, d), 5.26(1H, d), 6.77(1H, d), 6.94(1H, s), 7.29(1H, d), 7.55(1H, dd), 7.58(1H, d), 7.70(1H, dd), 7.81(1H, d), 8.05(1H, s), 8.35(1H, d), 8.55(1H, d).

Example B84

1-[4-Butyl-2-(methoxymethoxy)benzyl]isoquinoline

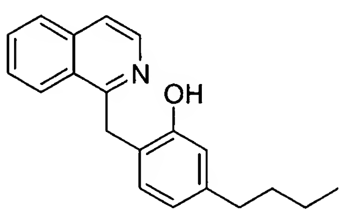


The title compound was obtained by treating the compound of Example B83 in the same manner as in Example B39.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.28-1.37(2H, m), 1.50-1.58(2H, m), 2.53(2H, t), 3.46(3H, s), 4.65(2H, s), 5.24(2H, s), 6.66(1H, dd), 6.89(1H, d), 6.92(1H, d), 7.51(1H, dd), 7.53(1H, d), 7.62(1H, dd), 7.79(1H, d), 8.23(1H, d), 8.47(1H, d).

Example B85

5-Butyl-2-(1-isoquinolylmethyl)phenol



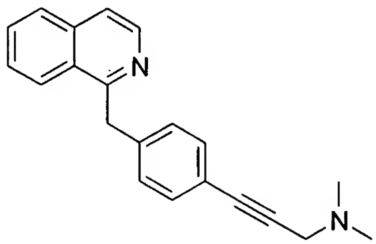
5 N hydrochloric acid (1.0 ml) was added to a solution of the compound of Example B84 (88 mg) in methanol (1.5 ml), and this reaction mixture was stirred at room temperature for 14 hours. The reaction mixture was neutralized with a 5 N aqueous sodium hydroxide solution, adjusted to pH 6.8 with phosphate buffer, and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give the title compound (44 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.23-1.37(2H, m), 1.48-1.60(2H, m), 2.51(2H, t), 4.56(2H, s), 6.65(1H, dd), 6.82(1H, d), 7.21(1H, d), 7.55(1H, d), 7.68(1H, dd), 7.72(1H, dd), 7.82(1H, d), 8.35(1H, d), 8.44(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B86

N-{3-[4-(1-isoquinolylmethyl)phenyl]-2-propynyl}-*N*,*N*-dimethylamine

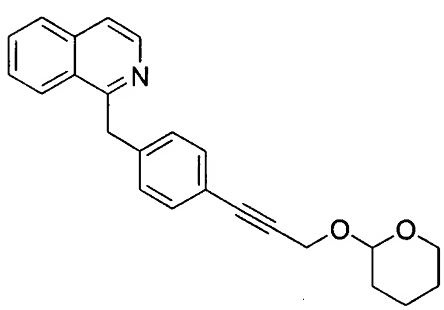


The title compound was obtained by treating the compound of Example B41 and 1-dimethylamino-2-propyne in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):2.04(3H, s), 2.34(3H, s), 3.47(2H,s), 4.66(2H, s), 7.20(2H, d), 7.32(2H, d), 7.53(1H, dd), 7.56(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.10(1H, d), 8.50(1H, d).

Example B87

1-{4-[3-(Tetrahydro-2*H*-2-pyranyloxy)-1-propynyl]benzyl}isoquinoline

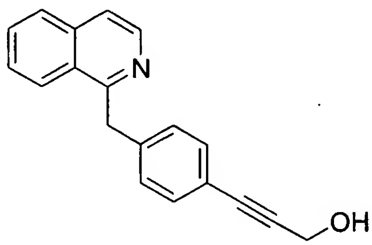


The title compound was obtained by treating the compound of Example B41 and tetrahydro-2-(2-propynyloxy)-2*H*-pyran in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.45-1.85(6H, m),3.50-3.60(1H, m), 3.84-3.90(1H, m), 4.42(1H, d), 4.48(1H, d), 4.66(2H, 8), 4.87(1H, dd), 7.15-7.21(2H, m), 7.33-7.36(2H, m), 7.50-7.70(3H, m), 7.81-7.86(1H, m), 8.07-8.10(1H, m), 8.48-8.51(1H, m).

Example B88

3-[4-(1-Isoquinolylmethyl)phenyl]-2-propyn-1-ol

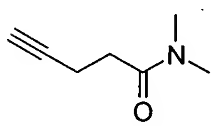


The title compound was obtained by treating the compound of Example B87 in the same manner as in Example B47.

^{1}H-NMR($CDCl_3$) δ (ppm):1.20-1.30(1H, m), 4.46(2H, s), 4.67(2H, s), 7.23(2H, d), 7.31(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.09(1H, d), 8.49(1H, d).

Example B89

N,N-dimethyl-4-pentynamide

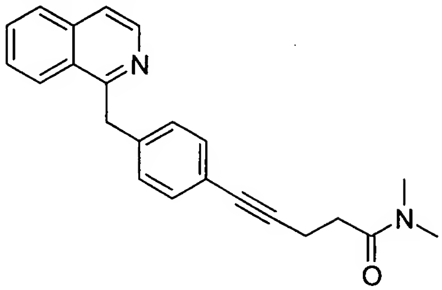


Dimethylamine (2 M solution in tetrahydrofuran, 8.53 ml), triethylamine (2.59 ml), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (3221 mg), were added to a solution of 4-pentynoic acid (552 mg) in methylene chloride (150 ml) and this reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was washed successively with 1 N hydrochloric acid, a saturated aqueous sodium hydrogencarbonate solution, water, and saturated brine, dried over anhydrous magnesium sulfate, then concentrated under reduced pressure to give the title compound (129 mg). The obtained compound was used in the following reaction without further purification.

^{1}H-NMR($CDCl_3$) δ (ppm):1.96-1.99(1H, m), 2.50-2.60(4H, m), 2.96(3H, s), 3.02(3H, s).

Example B90

N,*N*-dimethyl-5-[4-(1-isoquinolylmethyl)phenyl]-4-pentynamide

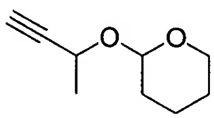


The title compound was obtained by treating the compound of Example B41 and the compound of Example B89 in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):2.59-2.64(2H, m), 2.71-2.75(2H, m), 2.96(3H, s), 3.03(3H, s), 4.66(2H, s), 7.18(2H, d), 7.28(2H, d), 7.43-7.70(3H, m), 7.90(1H, d), 8.09(1H, d), 8.50(1H, d).

Example B91

1-Methyl-2-propynyltetrahydro-2*H*-2-pyranyl ether



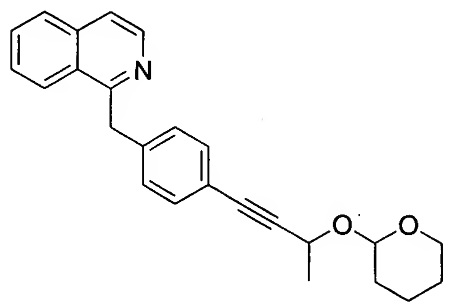
3,4-Dihydro-2*H*-pyran (7.15 ml) and pyridinium *p*-toluenesulfonate (2187 mg) were added to a solution of 3-butyn-2-ol (3051 mg) in dichloromethane (150 ml) , and this reaction mixture was stirred at room temperature for 29 hours.

The reaction mixture was washed successively with a saturated aqueous sodium hydrogencarbonate solution, water, and saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (4698 mg).

^{1}H-NMR($CDCl_3$).δ (ppm): 1.45(1.05H, d),1.48(1.95H, d), 1.50-1.90(6H, m), 2.37(0.65H, d), 2.43(0.35H, d), 3.50-3.60(1.3H, m), 3.80-3.86(0.7H, m),4.4-3-4.50(0.35H, m), 4.52-4.60(0.65H, m), 4.77(0.35H, t), 4.94(0.65H, t).

Example B92

1-{4-[3-(Tetrahydro-2*H*-2-pyranyloxy)-1-butynyl]benzyl}isoquinoline

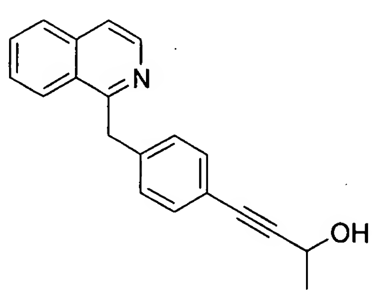


The title compound was obtained by treating the compound of Example B41 and the compound of Example B91 in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.40-1.80(6H, m), 1.49(1.05H, d), 1.52(1.95H, d), 3.49-3.60(1H, m), 3.80-3.88(0.65H, m), 3.99-4.06(0.35H, m), 4.65(2H, s), 4.74(1H, q), 4.83(0.35H, t), 4.97(0.65H, t), 7.18-7.22(2H, m), 7.32(2H, d), 7.54(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.08(1H, d), 8.49(1H, d).

Example B93

4-[4-(1-Isoquinolylmethyl)phenyl]-3-butyn-2-ol

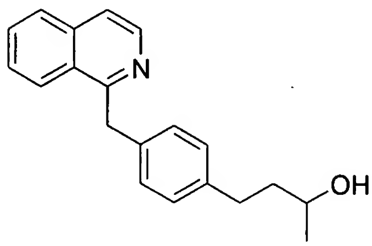


The title compound was obtained by treating the compound of Example B92 in the same manner as in Example B47.

^{1}H-NMR($CDCl_3$) δ (ppm):1.53(3H, d), 2.15(1H, brs), 4.68(2H, s), 4.72(1H, q), 7.21(2H, d), 7.31(2H, d), 7.54(1H, dd), 7.59(1H, d), 7.66(1H, dd), 7.84(1H, d), 8.10(1H, d), 8.51(1H, d).

Example B94

4-[4-(1-Isoquinolylmethyl)phenyl]-2-butanol

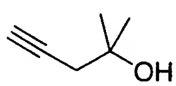


Treating the compound of Example B93 in the same manner as in Example B43, the obtained residue was separated and purified by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)] to give the title compound.

MS m/z(ESI:MH^+):292.2

Example B95

2-Methyl-4-pentyn-2-ol



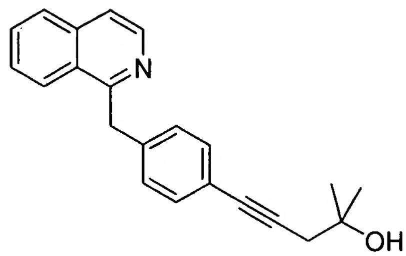
Lithium acetylide-ethylenediamine complex was added gradually to a mixed solution of isobutylene oxide (1889 mg) in tetrahydrofuran (13 ml) and dimethyl sulfoxide (20 ml) cooled to 0°C , and this reaction mixture was stirred at 0°C for 5 hours. After water was added, the reaction mixture was extracted with ethyl acetate, washed with saturated brine, dried over anhydrous magnesium sulfate, and then filtered through silica gel. The filtrate was concentrated under reduced pressure to give the title compound (3316 mg). This was used in the following reaction without further purification.

^{1}H-NMR($CDCl_3$) δ (ppm):1.33(6H, s), 2.09(1H, t), 2.38(2H, t).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B96

5-[4-(1-Isoquinolylmethyl)phenyl]-2-methyl-4-pentyn-2-ol

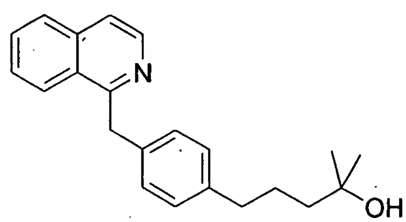


The title compound was obtained by treating the compound of Example B41 and the compound of Example B95 in the same manner as in Example B42.

^{1}H-NMR(DMSO-d6) δ (ppm):1.18(6H, s), 2.28(1H, s), 2.42(2H, s), 4.62(2H, s), 7.10-7.30(4H, m), 7.62(1H, dd), 7.71(1H, d), 7.72(1H, dd), 7.94(1H, d), 8.27(1H, d), 8.42(1H, d).

Example B97

5-[4-(1-Isoquinolylmethyl)phenyl]-2-methyl-2-pentanol

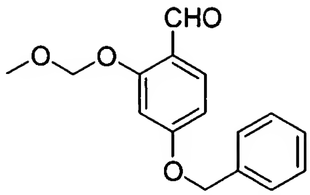


Treating the compound of Example B96 in the same manner as in Example B43, the obtained residue was separated and purified by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)] to give the title compound.

MS m/z(ESI:MH$^+$):320.2

Example B98

4-Benzyloxy-2-(methoxymethoxy)benzaldehyde

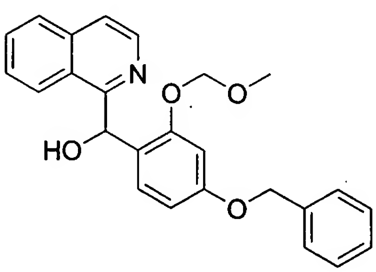


N,N-diisopropylethylamine (1.98 ml) and chloromethyl methyl ether (0.76 ml) were added to a solution of 4-benzyloxy-2-hydroxybenzaldehyde (2071 mg) in tetrahydrofuran (30 ml), and this reaction mixture was stirred and heated under reflux for 19 hours. *N,N*-diisopropylethylamine (2.7 ml) and chloromethyl methyl ether (1.04 ml) were further added, and the resulting mixture was stirred and heated under reflux for another 10 hours.
After water was added, the reaction mixture was extracted with ethyl acetate, washed with a saturated aqueous ammonium chloride solution and saturated brine, dried over anhydrous magnesium sulfate, then filtered through silica gel and alumina. The filtrate was concentrated under reduced pressure to give the title compound (2470 mg). This compound was used in the following reaction without further purification.

^{1}H-NMR($CDCl_3$) δ (ppm):3.52(3H, s), 5.12(2H, s), 5.27(2H, s), 6.68(1H, dd), 6.80(1H, d), 7.33-7.45(5H, m), 7.82(1H, d), 10.33(1H, s).

Example B99

[4-(Benzyloxy)-2-(methoxymethoxy)phenyl](1-isoquinolyl)methanol



The title compound was obtained by treating the compound of Example B98 in the same manner as in Example B82.

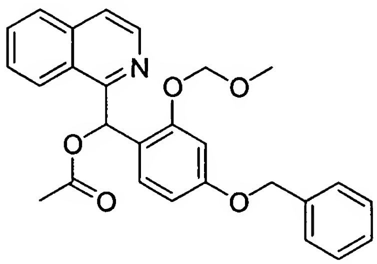
^{1}H-NMR(DMSO-d6) δ (ppm):3.16(3H, s), 5.01(2H, s), 5.11(1H, d), 5.14(1H, d), 6.59(1H, dd), 6.66-6.70(2H, m), 7.18(1H, d), 7.31(1H, d), 7.34-7.42(4H, m), 7.61(1H, dd), 7.71(1H, d), 7.75(1H, d), 7.95(1H, d),

8.28(1H, d), 8.43(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B100

[4-(Benzyloxy)-2-(methoxymethoxy)phenyl](1-isoquinolyl)methyl acetate

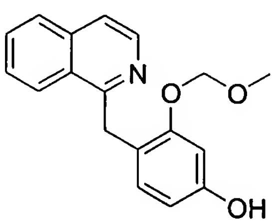


The title compound was obtained by treating the compound of Example B99 in the same manner as in Example B38.

^{1}H-NMR($CDCl_3$) δ (ppm):2.21(3H, s), 3.42(3H, s), 4.98(1H, d), 5.00(1H, d),5.21-5.27(2H, m), 6.54(1H, dd), 6.81(1H, d), 7.25(1H, d), 7.30-7.41(5H, m), 7.53(1H, dd), 7.57(1H, d), 7.63(1H, dd), 7.80(1H, d), 8.00(1H, s), 8.29(1H, d), 8.55(1H, d).

Example B101

4-(1-Isoquinolylmethyl)-3-(methoxymethoxy)phenol

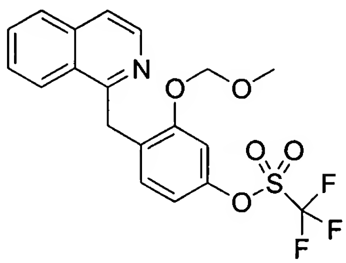


The title compound was obtained by treating the compound of Example B100 in the same manner as in Example B39.

^{1}H-NMR(DMSO-d6) δ (ppm):3.36(3H, s), 4.44(2H, s), 5.17(2H, s), 6.22(1H, d), 6.52(1H, s), 6.67(1H, d), 7.57-7.76(3H, m), 7.92(1H, d), 8.22(1H, d), 8.37(1H, d), 9.24(1H, brs).

Example B102

4-(1-Isoquinolylmethyl)-3-(methoxymethoxy)phenyl trifluoro-methanesulfonate

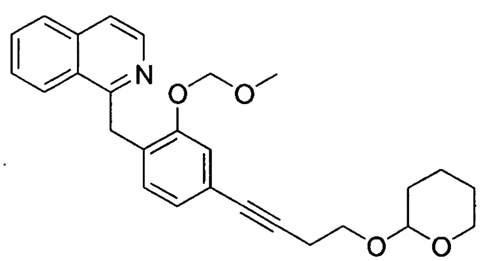


The title compound was obtained by treating the compound of Example B101 in the same manner as in Example B41.

^{1}H-NMR($CDCl_3$) δ (ppm):3.43(3H, s), 4.65(2H, s), 5.24(2H, s), 6.77(1H, dd), 7.04(1H, d), 7.07(1H, d), 7.54-7.61(2H, m), 7.67(1H, dd), 7.84(1H, d), 8.16(1H, d), 8.47(1H, d).

Example B103

1-{2-(Methoxymethoxy)-[4-(tetrahydro-2*H*-2-pyranyloxy)-1-butynyl]benzyl}isoquinoline

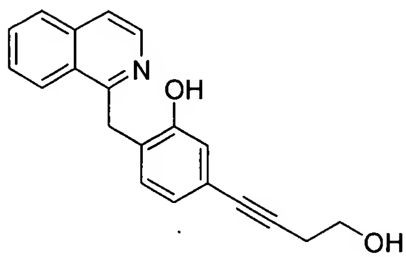


The title compound was obtained by treating the compound of Example B102 and 2-(3-butynyloxy)tetrahydro-2*H*-pyran in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.51-1.90(6H, m), 2.68(2H, t), 3.50(3H, s), 3.49-3.55(1H, m), 3.58-3.65(1H, m), 3.84-3.94(2H, m), 4.63-4.68(1H, m), 4.65(2H, s), 5.23(2H, s), 6.76(1H, dd), 7.04(1H, d), 7.07(1H, d), 7.49-7.69(3H, m), 7.81(1H, d), 8.14(1H, d), 8.47(1H, d).

Example B104

5-(4-Hydroxy-1-butynyl)-2-(1-isoquinolylmethyl)phenol



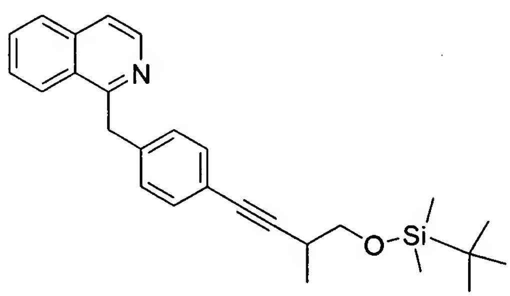
The title compound was obtained by treating the compound of Example B103 in the same manner as in Example B85.

^{1}H-NMR($CDCl_3$) δ (ppm):1.80(1H, brs), 2.66(2H, t), 3.73-3.82(2H, m), 4.58(2H, s), 6.87(1H, d), 7.04(1H, s), 7.23(1H, d), 7.60(1H, d), 7.69-7.78(2H, m), 7.86(1H, d), 8.37(1H, d), 8.42(1H, d).

The proton of the phenolic hydroxyl group was not observed in the NMR spectrum.

Example B105

1-(*t*-Butyl)-1,1-dimethylsilyl {4-[4-(1-isoquinolylmethyl)-phenyl]-2-methyl-3-butynyl} ether



Triphenylphosphine (18.37 g) was added to an ice-cooled solution of carbon tetrabromide (11.19 g) in methylene chloride (60 ml) , and this reaction mixture was stirred at that temperature for 1 hour. A solution of 3-{[1-(*t*-butyl)-1,1-dimethylsilyl]oxy}-2-methylpropanal, which was synthesized according to Tetrahedron Lett., 4347 (1979), in methylene chloride (14 ml) was added dropwise, and the resulting reaction mixture was further stirred for 1 hour. The reaction mixture was diluted with methylene chloride, washed successively with saturated aqueous sodium hydrogencarbonate solution, saturated an aqueous ammonium

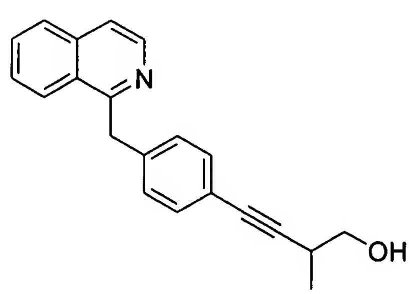
chloride solution and saturated brine, dried over magnesium sulfate, and then concentrated under reduced pressure. Ether was added to this residue, insoluble material was separated by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give *t*-butyl[(4,4-dibromo-2-methyl-3-butenyl)oxy]-dimethylsilane (2385 mg).

Next, a 2.47 M n-butyl lithium solution in hexane (3.15 ml) was added dropwise to a solution of *t*-butyl[(4,4-dibromo-2-methyl-3-butenyl)oxy]dimethylsilane (1326 mg) in tetrahydrofuran (10 ml) cooled to −70°C, and this mixture was stirred at that temperature for 1 hour. A saturated aqueous ammonium chloride solution was further added, and the resulting mixture was warmed to room temperature. After water was added, the reaction mixture was extracted with ether. The ether layer was washed with saturated brine, dried over anhydrous magnesium sulfate, then filtered through silica gel. The filtrate was concentrated under reduced pressure. The obtained residue and the compound of Example B41 were treated in the same manner as in Example B42 to obtain the title compound.

^{1}H-NMR($CDCl_3$) δ (ppm):0.07(6H, s), 0.90(9H, s), 1.18(3H, d),2.70-2.80(1H, m), 3.47(1H, dd), 3.70(1H, dd), 4.65(2H, s), 7.16(2H, d), 7.27(2H, d), 7.51(1H, dd), 7.56(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.07(1H, d), 8.49(1H, d).

Example B106

4-[4-(1-Isoquinolylmethyl)phenyl]-2-methyl-3-butyn-1-ol



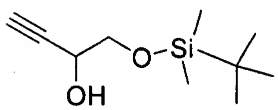
The title compound was obtained by treating the compound of Example

B105 in the same manner as in Example B47.

^{1}H-NMR(DMSO-d6) δ (ppm):1.11(3H, d), 2.60-2.70(1H, m), 3.28(1H, d), 3.44(1H, d), 4.58(2H, s), 4.85-4.90(1H, m), 7.23(4H, s), 7.61(1H, dd), 7.70(1H, d), 7.71(1H, dd), 7.93(1H, d), 8.25(1H, d), 8.42(1H, d).

Example B107

1-{[1-(*t*-Butyl)-1,1-dimethylsilyl]oxy}-3-butyn-2-ol

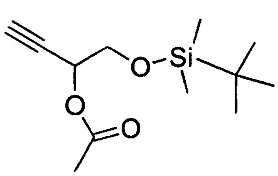


Ethynyl magnesium bromide in tetrahydrofuran (0.5 M, 90 ml) was added to anhydrous tetrahydrofuran (20 ml) cooled to -78°C under nitrogen atmosphere. A solution of *t*-butyldimethylsiloxyacetaldehyde (6000 mg) in tetrahydrofuran (30 ml) was added dropwise, and thee resulting mixture was stirred at -78°C for 45 minutes, warmed to room temperature, stirred for 1 hour 40 minutes, then cooled on ice. After a saturated aqueous ammonium chloride solution was added, the reaction mixture was extracted with ether, washed with water and saturated brine, dried over anhydrous magnesium sulfate, and then filtered through silica gel. The filtrate was concentrated under reduced pressure to give the title compound (8.55 g). This compound was used in the following reaction without further purification.

^{1}H-NMR($CDCl_3$) δ (ppm):0.08(6H, s), 0.91(9H, s), 2.43 (1H, d), 2.60-2.66(1H, m), 3.65-3.70(1H, m), 3.73-3.81(1H, m), 4.38-4.42(1H, m).

Example B108

1-{[1-(*t*-Butyl)-1,1-dimethylsilyl]oxy}methyl)-2-propynyl acetate

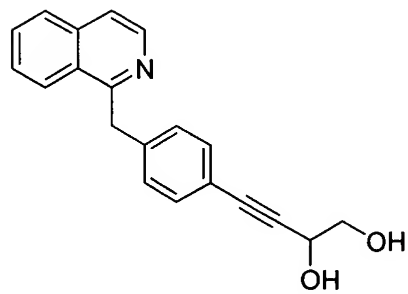


The title compound was obtained by treating the compound of Example B107 in the same manner as in Example B38.

^{1}H-NMR($CDCl_3$) δ (ppm):0.08(6H, s), 0.90(9H, s), 2.11(3H, s), 2.44(1H,d), 3.80-3.88(2H, m), 5.41-5.55(1H, m).

Example B109

4-[4-(1-Isoquinolylmethyl)phenyl]-3-butyn-1,2-diol

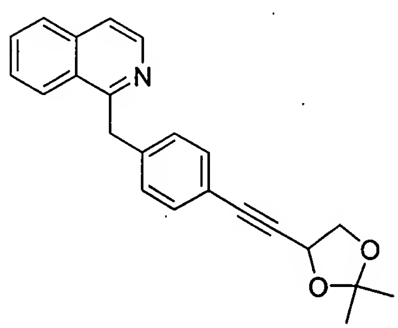


The compound of Example B41 and the compound of Example B108 were treated in the same manner as in Example B42 to give the coupling product. The title compound was obtained by deprotecting the hydroxyl protecting group of the coupling product in the same manner as in Example B47.

^{1}H-NMR(DMSO-d6) δ (ppm):3.40-3.45(1H, m), 3.70-3.82(1H, m), 4.30-4.35(1H, m), 4.63(2H, s), 4.90(1H, t), 5.46(1H, d), 7.25-7.30(4H, m), 7.62(1H, dd), 7.71(1H, d), 7.73(1H, dd), 7.94(1H, d), 8.28(1H, d), 8.43(1H, d).

Example B110

1-{4-[2-(2,2-Dimethyl-1,3-dioxolan-4-yl)-1-ethynyl]benzyl}-isoquinoline



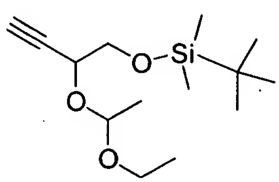
2,2-Dimethoxypropane (0.36 ml), 10-camphorsulfonic acid (43 mg), and molecular sieves (4 Å) were added to a solution of the compound of Example B109 (34 mg) in dimethylformamide (2 ml), and this reaction

mixture was stirred at 75°C for 9 hours. After an saturated aqueous sodium carbonate solution was added, the reaction mixture was extracted with ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (14 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):1.40(3H, s), 1.50(3H, s), 3.97(1H, dd), 4.21(1H, dd), 4.66(2H, s), 4.91(1H, dd), 7.19(2H, d), 7.32(2H, d), 7.52(1H, dd), 7.65-7.78(2H, m), 8.08(1H, d), 8.09(1H, d), 8.49(1H, d).

Example B111

t-Butyl{[2-(1-ethoxyethoxy)-3-butynyl]oxy}dimethylsilane

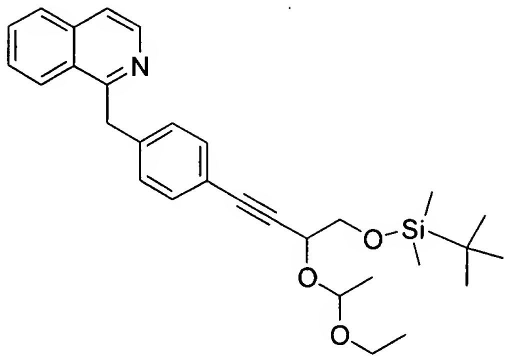


Ethyl vinyl ether (1.21 ml) and pyridinium *p*-toluenesulfonate (317 mg) were added to a solution of 1-{[1-(*t*-butyl)-1,1-dimethylsilyl]oxy}-3-butyn-2-ol (1687 mg) in methylene chloride (90 ml), and this mixture was stirred at room temperature for 1 hour. The methylene chloride layer was washed with a saturated aqueous sodium hydrogencarbonate solution and saturated brine, dried over anhydrous magnesium sulfate, then concentrated under reduced pressure to give the title compound (1962 mg). This compound was used in the following reaction without further purification.

^{1}H-NMR(DMSO-d6) δ (ppm):0.00(6H, s), 0.81(9H, s), 1.01-1.07(3H, m), 1.10-1.20(1H, m), 1.18(3H, d), 3.35-3.63(4H, m), 4.18-4.27(1H, m), 4.74(0.5H, q), 4.81(0.5H, q).

Example B112

1-{4-[4-{[1-(*t*-Butyl)-1,1-dimethylsilyl]oxy}-3-(1-ethoxyethoxy)-1-butynyl]benzyl}isoquinoline

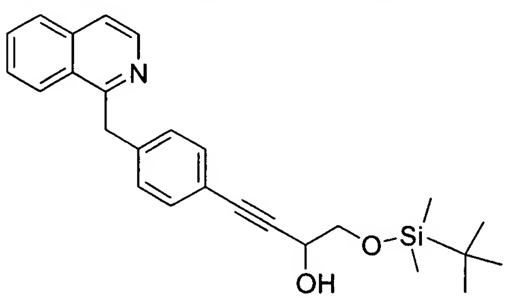


The title compound was obtained by treating the compound of Example B41 and the compound of Example B111 in the same manner as in Example B42.

^{1}H-NMR(DMSO-d6) δ (ppm):0.00(6H, s), 0.80(9H, s), 1.01-1.05(3H, m), 1.19(3H, d), 3.39-3.70(4H, m), 4.41(0.5H, t), 4.48(0.5H, t), 4.59(2H, s), 4.79(0.5H, q), 4.87(0.5H, q), 7.20-7.30(4H, m), 7.58(1H, dd), 7.68(1H, d), 7.69(1H, dd), 7.91(1H, d), 8.24(1H, d), 8.38(1H, d).

Example B113

1-{[1-(*t*-Butyl)-1,1-dimethylsilyl]oxy}4-[4-(1-isoquinolyl-methyl)phenyl]-3-butyn-2-ol

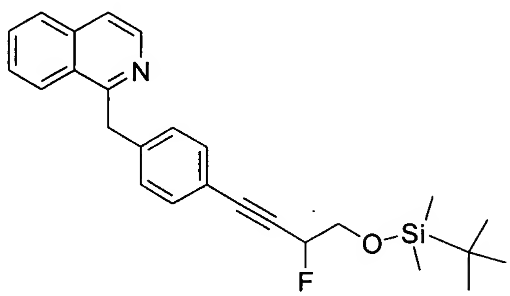


Pyridinium *p*-toluenesulfonate (486 mg) was added to a solution of the compound of Example B112 (474 mg) in methanol (15 ml) , and this reaction mixture was stirred at room temperature for 24 hours. After ethyl acetate was added, the reaction mixture was washed with a saturated aqueous sodium hydrogencarbonate solution and saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (265 mg).

^{1}H-NMR(DMSO-d6) δ (ppm):0.01(6H, s), 0.82(9H, s), 3.55-3.62(2H, m), 4.30-4.39(1H, m), 4.61(2H, s), 5.51(1H, d), 7.20-7.27(4H, m), 7.50-7.63(1H, m), 7.67-7.74(2H, m), 7.92(1H, d), 8.27(1H, d), 8.41(1H, d).

Example B114

1-(*t*-Butyl)-1,1-dimethylsilyl{2-fluoro-4-[4-(1-isoquinolyl-methyl)phenyl]-3-butynyl} ether

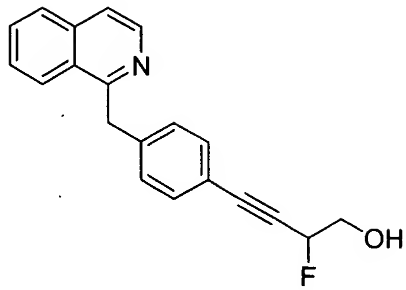


A solution of the compound of Example B113 (116 mg) in methylene chloride (2 ml) was added dropwise to a solution of (diethylamino)sulfur trifluoride (44 μl) in methylene chloride (2 ml) cooled to -78°C under nitrogen atmosphere. Upon stirring for 15 minutes, the reaction mixture was stirred at room temperature for another 8 hours. A saturated aqueous sodium hydrogencarbonate solution was added, the resulting reaction mixture was extracted with methylene chloride. The methylene chloride layer was washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (42 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.10(6H, s), 0.91(9H, s), 3.83-4.00(2H, m), 4.67(2H, s), 5.17(1H, ddd), 7.22(2H, d), 7.34(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.08(1H, d), 8.50(1H, d).

Example B115

2-Fluoro-4-[4-(1-isoquinolylmethyl)phenyl]-3-butyn-1-ol

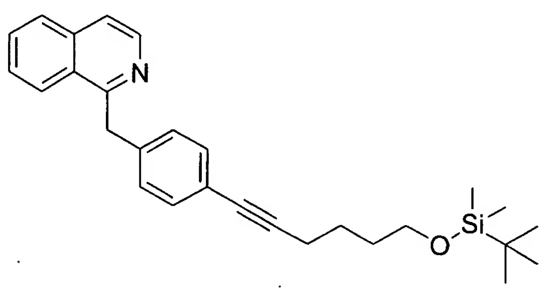


The title compound was obtained by treating the compound of Example B114 in the same manner as in Example B47.

^{1}H-NMR($CDCl_3$) δ (ppm):1.31(1H, brs), 3.77-3.95(2H, m), 4.67(2H, s), 5.35(1H, ddd), 7.22(2H, d), 7.35(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.07(1H, d), 8.50(1H, d).

Example B116

1-(*t*-Butyl)-1,1-dimethylsilyl {6-[4-(1-isoquinolylmethyl)-phenyl]-5-hexynyl} ether

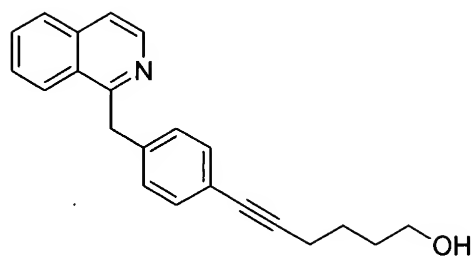


The title compound was obtained by treating the compound of Example B41 and *t*-butyl(5-hexynyloxy)dimethylsilane in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):0.04(6H, s), 0.88(9H, s), 1.55-1.70(4H, m), 2.39(2H, t), 3.64(2H, t), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.51(1H, dd), 7.55(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.08(1H, d), 8.49(1H, d).

Example B117

6-[4-(1-Isoquinolylmethyl)phenyl]-5-hexyn-1-ol



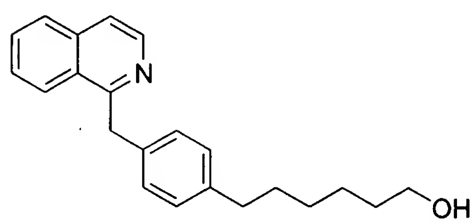
The title compound was obtained by treating the compound of Example B116 in the same manner as in Example B47.

^{1}H-NMR($CDCl_3$) δ (ppm):1.60-1.80(4H, m), 2.42(2H, t), 3.69(2H, t), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.08(1H, d), 8.49(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B118

6-[4-(1-Isoquinolylmethyl)phenyl]-1-hexanol

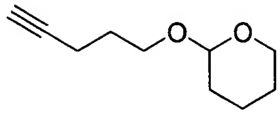


Treating the compound of Example B117 in the same manner as in Example B43, the obtained residue was separated and purified by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)] to give the title compound.

MS m/z(ESI:MH^+):320.2

Example B119

2-(4-Pentynyloxy)tetrahydro-2*H*-pyran

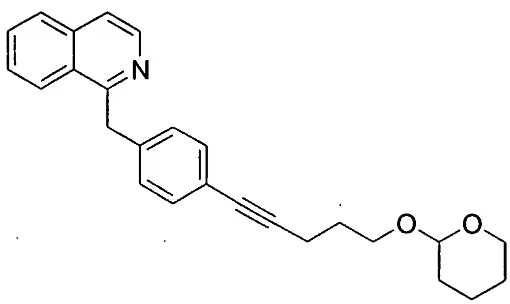


The title compound was obtained by treating 4-pentyn-1-ol in the same manner as in Example B91.

^{1}H-NMR($CDCl_3$) δ (ppm):1.50-1.90(8H, m), 1.95(1H, t), 2.30-2.35(2H, m), 3.46-3.54(2H, m), 3.80-3.90(2H, m), 4.60(1H, dd).

Example B120

1-{4-[5-(Tetrahydro-2*H*-2-pyranyloxy)-1-pentynyl]benzyl}-isoquinoline

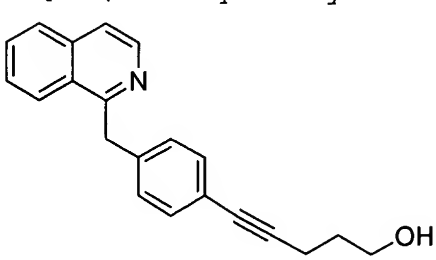


The title compound was obtained by treating the compound of Example B41 and the compound of Example B119 in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.49-1.90(8H, m), 2.49(2H, t), 3.47-3.54(2H, m), 3.82-3.90(2H, m), 4.60(1H, dd), 4.65(2H, s), 7.17(2H, d), 7.27(2H, d), 7.52(1H, dd), 7.58(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

Example B121

5-[4-(1-Isoquinolylmethyl)phenyl]-4-pentyn-1-ol



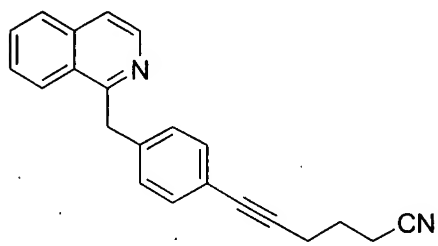
The title compound was obtained by treating the compound of Example B120 in the same manner as in Example B47.

^{1}H-NMR($CDCl_3$) δ (ppm):1.80-1.88(2H, m), 2.51(2H, t), 3.80(2H, t), 4.65(2H, s), 7.18(2H, d), 7.29(2H, d), 7.52(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B122

5-[4-(1-Isoquinolylmethyl)phenyl]-4-pentynylcyanide

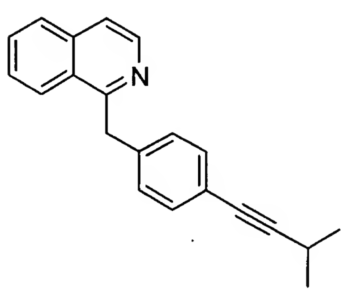


The title compound was obtained by treating the compound of Example B41 and 5-cyano-1-pentyne in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.85-1.98(2H, m), 2.40-2.60(4H, m), 4.66(2H, s), 7.20(2H, d), 7.28(2H, d), 7.53(1H, dd), 7.58(1H, d), 7.65(1H, dd), 7.83(1H, d), 8.09(1H, d), 8.50(1H, d).

Example B123

1-[4-(3-Methyl-1-butynyl)benzyl]isoquinoline



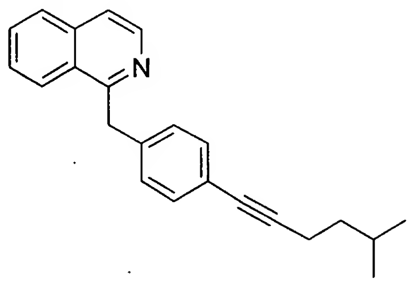
The title compound was obtained by treating the compound of Example B41 and 3-methyl-1-butyne in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.23(6H, d), 2.70-2.78(1H, m), 4.65(2H, s), 7.18(2H, d), 7.28(2H, d), 7.51(1H, dd), 7.58(1H, d), 7.64(1H, dd),

7.82(1H, d), 8.08(1H, d), 8.50(1H, d).

Example B124

1-[4-(5-Methyl-1-hexynyl)benzyl]isoquinoline

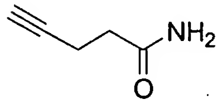


The title compound was obtained by treating the compound of Example B41 and 5-methyl-1-hexyne in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):0.91(6H, d), 1.47(2H, dt), 1.68-1.77(1H, m), 2.37(2H, t), 4.65(2H, s), 7.17(2H, d), 7.28(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.81(1H, d), 8.09(1H, d), 8.49(1H, d).

Example B125

4-Pentynamide

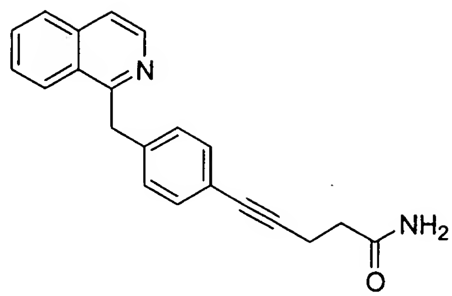


1-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (6775 mg) and ammonium hydrogencarbonate (5905 mg) were added to a solution of 4-pentynoic acid (2446 mg) in chloroform (75 ml), and this reaction mixture was stirred at room temperature for 17.5 hours. The reaction mixture was filtered through celite and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (249 mg).

^{1}H-NMR(DMSO-d6) δ (ppm):2.21(2H, t), 2.29-2.33(2H, m), 2.73(1H, t), 6.78-6.88(1H, m), 7.28-7.38(1H, m).

Example B126

5-[4-(1-Isoquinolylmethyl)phenyl]-4-pentynamide

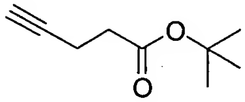


The title compound was obtained by treating the compound of Example B41 and the compound of Example B125 in the same manner as in Example B42.

^{1}H-NMR(DMSO-d6) δ (ppm):2.51(2H, t), 2.85(2H, t), 3.70(2H, brs), 4.59(2H, s), 7.05(2H, d), 7.23(2H, d), 7.61(1H, dd), 7.70(1H, d), 7.72(1H, dd), 7.94(1H, d), 8.30(1H, d), 8.43(1H, d).

Example B127

t-Butyl 4-pentynoate

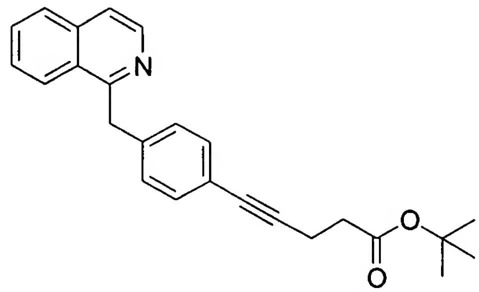


Benzyltriethylammonium chloride (5.92 g), potassium carbonate (93.4 g), and *t*-butyl bromide (143 ml) were added to a solution of 4-pentynoic acid (2550 mg) in *N,N*-dimethylacetamide (230 ml), and this reaction mixture was stirred at 55°C for 24 hours. After water was added, the reaction mixture was extracted with ethyl acetate, washed with water, dried over anhydrous magnesium chloride, and then filtered through silica gel. The filtrate was concentrated under reduced pressure to give the title compound (2.10 g). This compound was used in the following reaction without further purification.

^{1}H-NMR($CDCl_3$) δ (ppm):1.46(9H, s), 1.96-1.97(1H, m), 2.45-2.47(4H, m).

Example B128

t-Butyl 5-[4-(1-isoquinolylmethyl)phenyl]-4-pentynoate

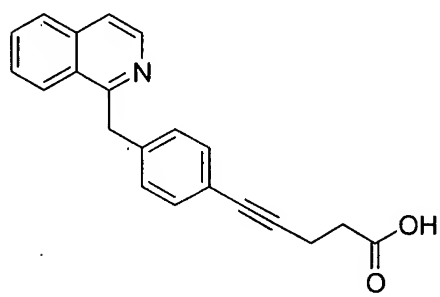


The title compound was obtained by treating the compound of Example B41 and the compound of Example B127 in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.45(9H, s), 2.49(2H, t), 2.64(2H, t), 4.64(2H, s), 7.21(2H, d), 7.26(2H, d), 7.52(1H, dd), 7.57(1H, d), 7.64(1H, dd), 7.82(1H, d), 8.09(1H, d), 8.49(1H, d).

Example B129

5-[4-(1-Isoquinolylmethyl)phenyl]-4-pentynoic acid



Treating the compound of Example B128 in the same manner as in Example B69, the obtained residue was separated and purified by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)] to give the title compound.

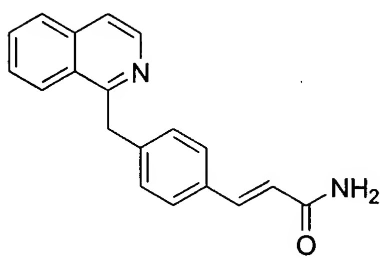
MS m/z(ESI:MH^+):316.1

The following compounds were synthesized as follows. That is, the title compound was obtained by reacting the compound of Example B41 with various reactants described below, according to Example B33. The various reactants are acrylamide, *N,N*-dimethylacrylamide, *t*-butyl

acrylate, and methyl vinyl sulfone. Furthermore, the coupling product obtained in this manner was subjected to either the reduction according to Example B39 or the deprotection of *t*-butyl ester according to Example B40, or both. The resulting product was purified by silica gel column chromatography or by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)].

Example B130

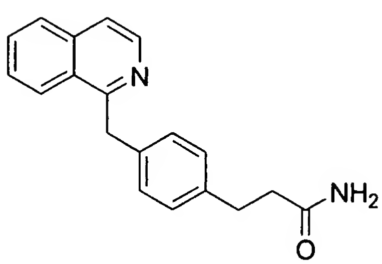
(*E*)-3-[4-(1-isoquinolylmethyl)phenyl]-2-propenamide



MS m/z(ESI:MH^+):289.3

Example B131

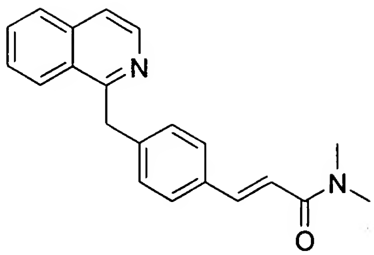
3-[4-(1-Isoquinolylmethyl)phenyl]-2-propanamide



MS m/z(ESI:MH^+):291.2

Example B132

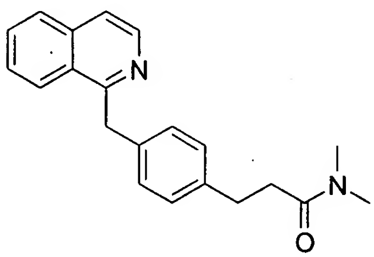
N,*N*-dimethyl-(*E*)- 3-[4-(1-isoquinolylmethyl)phenyl]-2-propenamide



MS m/z(ESI:MH+):317.3

Example B133

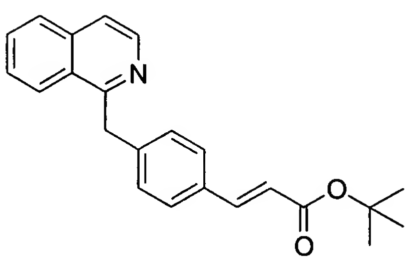
N,N-dimethyl-3-[4-(1-isoquinolylmethyl)phenyl]propanamide



MS m/z(ESI:MH+):319.1

Example B134

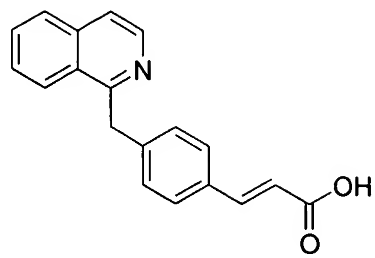
t-Butyl (*E*)-3-[4-(1-isoquinolylmethyl)phenyl]-2-propenoate



^{1}H-NMR($CDCl_3$) δ (ppm):1.51(9H, s), 4.68(2H, s), 6.28(1H, d), 7.27(2H, d), 7.39(2H, d), 7.49-7.60(3H, m), 7.65(1H, dd), 7.82(1H, d), 8.11(1H, d), 8.50(1H, d).

Example B135

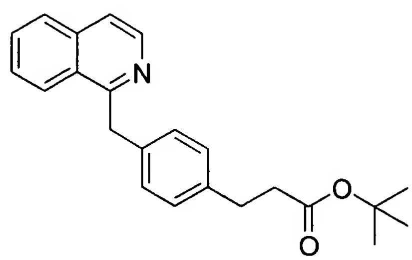
(*E*)-3-[4-(1-isoquinolylmethyl)phenyl]-2-propenoic acid



MS m/z(ESI:MH$^+$):290.2

Example B136

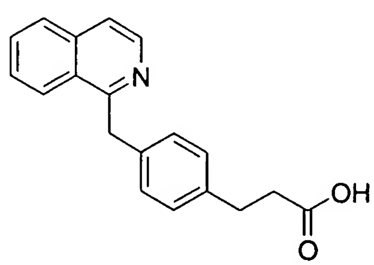
t-Butyl 3-[4-(1-isoquinolylmethyl)phenyl]propanoate



^{1}H-NMR($CDCl_3$) δ (ppm):1.37(9H, s), 2.47(2H, t), 2.83(2H, t), 4.64(2H, s), 7.07(2H, d), 7.19(2H, d), 7.52(1H, dd), 7.56(1H, d), 7.63(1H, dd), 7.81(1H, d), 8.14(1H, d), 8.49(1H, d).

Example B137

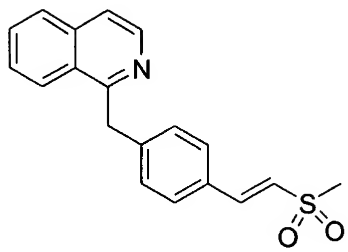
3-[4-(1-Isoquinolylmethyl)phenyl]propanoic acid



MS m/z(ESI:MH$^+$):292.1

Example B138

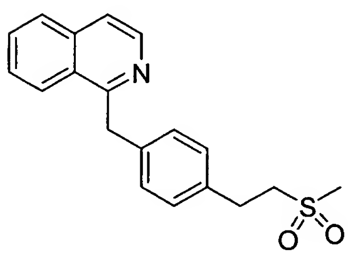
(*E*)-2-[4-(1-isoquinolylmethyl)phenyl]-1-ethenyl methylsulfone



MS m/z(ESI:MH^+):324.1

Example B139

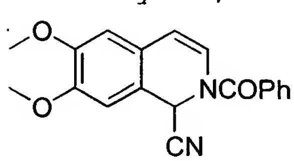
1-{4-[2-(Methylsulfonyl)ethyl]benzyl}isoquinoline



MS m/z(ESI:MH^+):326.1

Example B140

2-Benzoyl-6,7-dimethoxy-1,2-dihydro-1-isoquinolinecarbonitrile



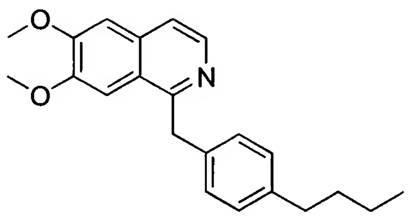
An aqueous potassium cyanide (1.0 g, 16 mmol) solution (2.3 ml) and benzoyl chloride (1.1 ml, 9.5 mmol) were added to a solution of 6,7-dimethoxyisoquinoline (1.0 g, 5.3 mmol), which was synthesized according to Tetrahedron, 37 (23), 3977 (1981), in methylene chloride (6.0 ml), and this reaction mixture was stirred while heating under reflux for 2 hours. The reaction mixture was cooled to room temperature, filtered through celite, and washed with methylene chloride and water. After the obtained filtrate was separated, the methylene chloride layer was washed successively with water, 2 N hydrochloric acid, water, and 2 N sodium hydroxide, dried over anhydrous magnesium sulfate, and then

concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (573 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):3.92(3H, s), 3.94(3H, s), 5.99(1H, d), 6.51-6.55(2H, m), 6.73(1H, s), 6.85(1H, s), 7.45-7.49(2H, m), 7.53-7.56(1H, m), 7.58-7.61(2H, m)

Example B141

1-(4-Butylbenzyl)-6,7-dimethoxyisoquinoline

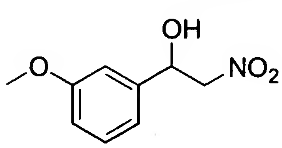


The title compound was obtained by treating the compound of Example B140 and the compound of Example B1 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.27-1.36(2H, m), 1.51-1.58(2H, m), 2.54(2H, t), 3.88(3H, s), 4.01(3H, s), 4.57(2H, s), 7.05(1H, s), 7.07(2H, d), 7.19(2H, d), 7.32(1H, s), 7.43(1H, d), 8.37(1H, d)

Example B142

1-(3-Methoxyphenyl)-2-nitro-1-ethanol



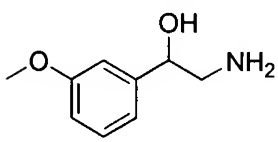
An aqueous sodium hydroxide solution (1.5 g of sodium hydroxide (37 mmol) was dissolved in 15 ml of water) was added dropwise to a solution of *m*-anisaldehyde (5.0 g, 37 mmol) and nitromethane (4.0 ml, 73 mmol) in methanol (50 ml) keeping the temperature of the solution at not higher than 30°C. The reaction mixture was then stirred at room temperature for 4 hours. Upon cooling on ice, an aqueous acetic acid solution (glacial acetic acid (37 mmol) was dissolved in 250 ml of water) was added, the resulting reaction mixture was extracted with ethyl acetate.

The ethyl acetate layer was washed successively with water and a 5% aqueous sodium hydrogencarbonate solution, dried over anhydrous magnesium sulfate, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (6.09 g).

^{1}H-NMR($CDCl_3$) δ (ppm):3.83(3H, s), 4.52(1H, dd), 4.61(1H, dd), 4.76-4.78(1H, m), 5.44-5.48(1H, m), 6.90(1H, dd), 6.96-6.98(2H, m), 7.25-7.34(1H, m)

Example B143

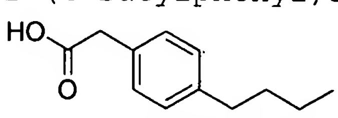
2-Amino-1-(3-methoxyphenyl)-1-ethanol



Palladium-carbon (10%, 0.64 g) and ammonium formate (4.8 g) were added to a mixed solution of the compound of Example B142 (3.0 g, 15 mmol) in tetrahydrofuran (43 ml) and methanol (43 ml), and this mixture was stirred at room temperature for 18 hours. The catalyst was removed by filtration, the filtrate was diluted with ether, precipitates were removed by filtration, and the obtained filtrate was concentrated to give the title compound (1.82 g). This compound was used in the following reaction without further purification.

Example B144

2-(4-Butylphenyl)acetic acid



Thionyl chloride (4.7 ml, 66 mmol) was added dropwise to a solution of 4-*n*-butylbenzyl alcohol (9.6 g, 59 mmol) in ether (120 ml), and this mixture was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure, and excess thionyl chloride was removed by azeotropic distillation with benzene. The residue was dissolved in dimethyl sulfoxide (50 ml), sodium cyanide (86 g, 1.8 mol) and

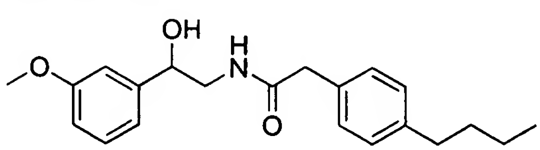
n-tetrabutylammonium iodide (2.2g, 5.9 mmol) were added to this solution, and the resulting mixture was stirred at room temperature for 16 hours. Water was added, and this mixture was extracted with ethyl acetate. The ethyl acetate layer was washed successively with water and saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give n-butylphenylacetonitrile (8.2 g) as a yellow oil. Next, concentrated sulfuric acid (48 ml) was added dropwise to water (58 ml), this solution was cooled to 50°C, and n-Butylphenylacetonitrile (8.2 g) obtained above was added dropwise to the solution. The resulting mixture was stirred while heating under reflux for 16 hours. Upon cooling to room temperature, the precipitated crystals were collected by filtration, washed with water, and dissolved in a 0.1 N aqueous sodium hydroxide solution (200 ml). Norit (5 g) was added, and this mixture was stirred and refluxed for 2 hours. After Norit was removed by filtration through celite, the filtrate was cooled to room temperature and acidified with 1 N hydrochloric acid to precipitate crystals. The precipitated crystals were collected by filtration, washed with water, and dried to give the title compound (3.5 g).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.93(3H, t), 1.30-1.40(2H, m), 1.53-1.62(2H, m), 2.59(2H, t), 3.62(2H, s), 7.15(2H, d), 7.20(2H, d)

The OH of the carboxyl group was not observed in the NMR spectrum.

Example B145

N-[2-Hydroxy-2-(3-methoxyphenyl)ethyl]-2-(4-butylphenyl)-acetamide



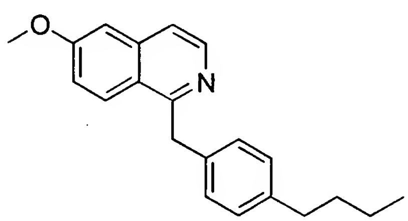
Thionyl chloride (0.76 ml, 10 mmol) was added to a solution of the compound of Example B144 (1.0 g, 5.2 mmol) in benzene (10 ml), and the mixture was stirred under reflux for 2 hours. Upon concentration,

excess thionyl chloride was removed by azeotropic distillation with benzene. The obtained residue and the compound of Example B143 (0.87 g, 5.2 mmol) were dissolved in ether (5 ml), an aqueous sodium hydroxide solution (0.21 g of sodium hydroxide was dissolved in 4.2 ml of water) was added thereto, and the mixture was stirred vigorously at room temperature for 30 minutes. The ether layer was separated and concentrated under reduced pressure to give the title compound (600 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.94(3H, t), 1.31-1.40(2H, m), 1.57-1.63(2H, m), 2.60(2H, m), 3.30-3.37(1H, m), 3.56(2H, s), 3.60-3.66(1H, m), 3.80(3H, s), 3.81(1H, d), 4.79-4.81(1H, m), 6.80-6.89(3H, m), 7.10(2H, d), 7.16(2H, d), 7.20-7.25(1H, m)

Example B146

1-(4-Butylbenzyl)-6-methoxyisoquinoline

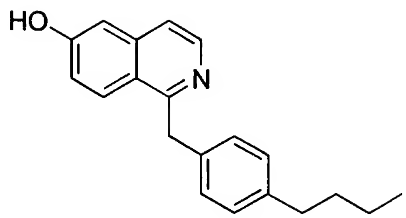


Phosphorus oxychloride (1.6 ml) was added to a solution of the compound of Example B145 (600 mg, 1.7 mmol) in acetonitrile (15 ml), and the mixture was stirred under reflux for 1 hour 30 minutes. The mixture was cooled on ice, made alkaline with a 5% aqueous sodium hydrogencarbonate solution, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (82 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.53(2H, t), 3.92(3H, s), 4.57(2H, s), 7.05-7.07(3H, m), 7.13-7.18(3H, m), 7.45(1H, d), 8.06(1H, d), 8.41(1H, d)

Example 147

1-(4-Butylbenzyl)-6-isoquinolinol

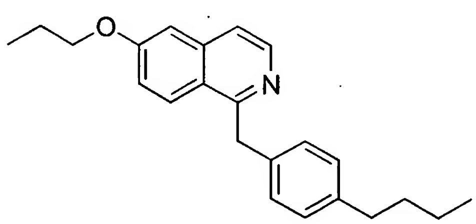


A 47% hydrobromic acid solution was added to the compound of Example B146 (82 mg), and the mixture was stirred under reflux for 19 hours. The mixture was concentrated under reduced pressure, water was added, and the resulting mixture was neutralized with sodium carbonate to precipitate crystals. The obtained crystals were collected by filtration, washed with water, and then dried to give the title compound (74 mg).

^{1}H-NMR(CD_3OD) δ (ppm):0.89(3H, t), 1.25-1.34(2H, m), 1.49-1.57(2H, m), 2.52(2H, t), 4.63(2H, s), 7.03-7.13(6H, m), 7.49(1H, d), 8.10(1H, d), 8.18(1H, d)

Example B148

1-(4-Butylbenzyl)-6-propoxyisoquinoline



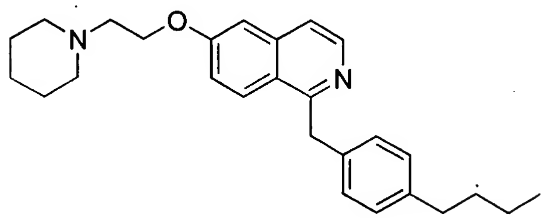
Silver carbonate (40 mg, 0.14 mmol) was added to a solution of the compound of Example B147 (20 mg, 0.069 mmol) and 1-iodopropane (0.4 ml, 4.1 mmol) in toluene (1.0 ml) , and the mixture was stirred in the dark at 50°C for 4 hours. Upon cooling to room temperature, the mixture was filtered through celite and washed with a mixed solution of toluene and methanol (9:1). The obtained filtrate was concentrated under reduced pressure, and the resulting residue was purified by silica gel column chromatography to give the title compound (13 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.08(3H, t), 1.30-1.33(2H, m), 1.51-1.57(2H, m), 1.86-1.91(2H, m), 2.54(2H, t), 4.05(2H, t), 4.58(2H,

s), 7.05-7.07(3H, m), 7.14-7.18(3H, m), 7.43-7.44(1H, m), 8.05-8.07(1H, m), 8.40-8.41(1H, m)

Example B149

1-(4-Butylbenzyl)-6-(2-piperidinoethoxy)isoquinoline

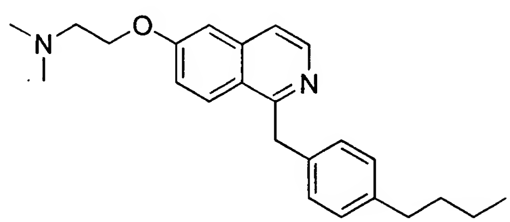


The title compound was obtained in the same manner as in Example 148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.46-1.57(8H, m), 2.50-2.54(6H, m), 2.83-2.86(2H, m), 4.23(2H, t), 4.56(2H, s), 7.04-7.06(3H, m), 7.13-7.17(3H, m), 7.43(1H, d), 8.04(1H, d), 8.40(1H, d)

Example B150

N-({[1-(4-butylbenzyl)-6-isoquinolyl]oxy}ethyl)-*N*,*N*-dimethyl-amine

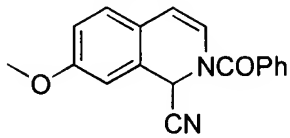


The title compound was obtained in the same manner as in Example 148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.49-1.57(2H, m), 2.37(6H, s), 2.52(2H, t), 2.80(2H, t), 4.19(2H, t), 4.57(2H, s), 7.04-7.06(3H, m), 7.15-7.19(3H, m), 7.43(1H, d), 8.05(1H, d), 8.40(1H, d)

Example B151

2-Benzoyl-7-methoxy-1,2-dihydro-1-isoquinolinecarbonitrile

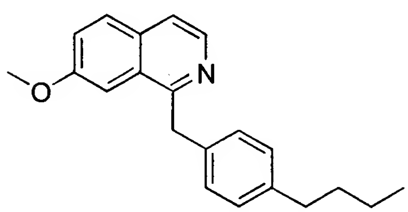


The title compound was obtained by treating 7-methoxyisoquinoline, which was synthesized according to Tetrahedron, 27, 1253 (1971), in the same manner as in Example B140.

^{1}H-NMR($CDCl_3$) δ (ppm):3.87(3H, s), 6.03(1H, brd), 6.56-6.54(2H, m), 6.90(1H, s), 6.95(1H, dd), 7.17(1H, d), 7.46-7.50(2H, m), 7.54-7.62(3H, m)

Example B152

1-(4-Butylbenzyl)-7-methoxyisoquinoline

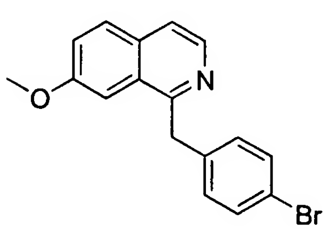


The title compound was obtained by treating the compound of Example B1 and the compound of Example B151 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.56-1.58(2H, m), 2.55(2H, t), 3.82(3H, s), 4.59(2H, s), 7.07(2H, d), 7.20(2H, d), 7.26-7.29(1H, m), 7.35(1H, d), 7.49(1H, d), 7.70(1H, d), 8.38-8.40(1H, m)

Example B153

1-(4-Bromobenzyl)-7-methoxyisoquinoline

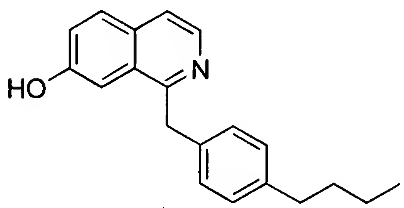


The title compound was obtained by treating the compound of Example B31 and the compound of Example B151 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):3.84(3H, s), 4.57(2H, s), 7.14-7.16(2H, m), 7.26(1H, s), 7.29-7.32(1H, m), 7.37-7.39(2H, m), 7.51(1H, d), 7.73(1H, d), 8.39(1H, d)

Example B154

1-(4-Butylbenzyl)-7-isoquinolinol



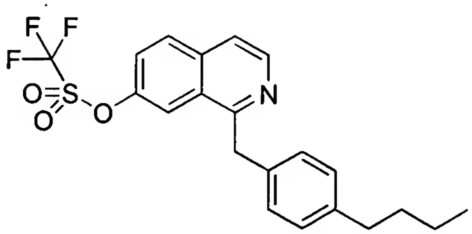
The title compound was obtained by treating the compound of Example B152 in the same manner as in Example B147.

^{1}H-NMR(DMSO-d_6) δ (ppm):0.83(3H, t), 1.21-1.26(2H, m), 1.44-1.48(2H, m), 4.68(2H, s), 7.11(2H, d), 7.18(2H, d), 7.59-7.62(2H, m), 8.10-8.17(2H, m), 8.38(1H, d), 10.9(1H, brs)

(The two methylene protons of the butyl group overlapped with the DMSO signal and could not be observed.)

Example B155

1-(4-Butylbenzyl)-7-isoquinolyl trifluoromethanesulfonate



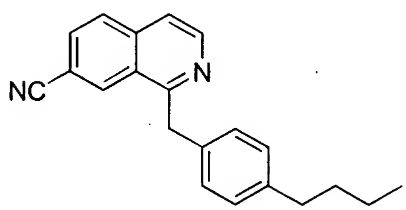
4-Nitrophenol triflate (0.72 g, 2.7 mmol), which was synthesized according to J. Org. Chem., 64, 7638 (1999), and potassium carbonate (1.1 g, 8.1 mmol) were added to a solution of the compound of Example B154 (1.0 g, 2.7 mmol) in dimethylformamide (30 ml), and the mixture

was stirred at room temperature for 2 hours. After water was added, the resulting mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with 1 N sodium hydroxide and saturated brine, dried over magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (1.0 g).

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.27-1.37(2H, m), 1.51-1.59(2H, m), 2.54(2H, t), 5.10(2H, s), 6.38(1H, s), 6.95(2H, d), 7.04(2H, d), 7.44(1H, d), 7.55(1H, d), 7.75(1H, d), 8.45(1H, d)

Example B156

1-(4-Butylbenzyl)-7-isoquinolinecarbonitrile

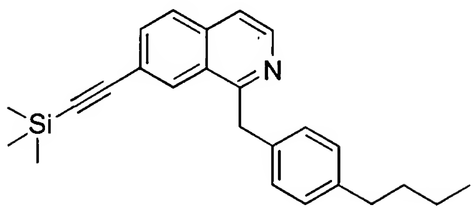


Zinc cyanide (215 mg, 1.8 mmol), tetrakis(triphenylphosphine)palladium (41 mg, 0.035 mmol), and lithium chloride (120 mg, 2.8 mmol) were added to a solution of the compound of Example B155 (400 mg, 0.95 mmol) in dimethylformamide (2 ml) under nitrogen atmosphere, and the mixture was stirred at 120°C for 2 hours. After cooling to room temperature, saturated sodium hydrogencarbonate was added, and the resulting mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with saturated brine, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (71 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.26-1.35(2H, m), 1.47-1.55(2H, m), 2.50(2H, t), 4.91(2H, s), 6.97(2H, d), 7.07(2H, d), 7.28-7.31(1H, m), 7.42(1H, d), 7.51(1H, d), 7.74(1H, d), 8.34(1H, d)

Example B157

1-(4-Butylbenzyl)-7-[2-(1,1,1-trimethylsilyl)-1-ethynyl]-isoquinoline

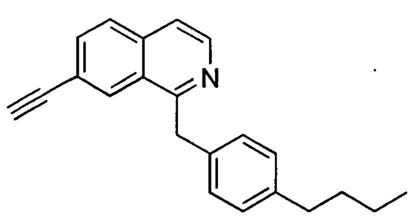


Palladium acetate (11 mg, 0.047 mmol), 1,1′-bis(diphenylphosphino)ferrocene (72 mg, 0.13 mmol), and lithium chloride (25 mg, 0.59 mmol) were added to a solution of the compound of Example B155 (100 mg, 0.24 mmol) and trimethylsilylacetylene (65 μl, 0.47 mmol) in dimethylformamide (3.0 ml), and the reaction system was purged with nitrogen. Triethylamine (59 μl, 0.43 mmol) and copper iodide (2 mg, 0.018 mmol) were added, and the resulting mixture was stirred at 80°C for 21 hours, then cooled to room temperature. After water and ethyl acetate were added for partition, the ethyl acetate layer was washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (7.0 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.28-0.32(9H, m), 0.92(3H, t), 1.32-1.38(2H, m), 1.54-1.57(2H, m), 2.57(2H, t), 4.63(2H, s), 7.10(2H, d), 7.20(2H, d), 7.52(1H, d), 7.67-7.69(1H, m), 7.75(1H, d), 8.34(1H, d), 8.51(1H, d)

Example B158

1-(4-Butylbenzyl)-7-(1-ethynyl)isoquinoline



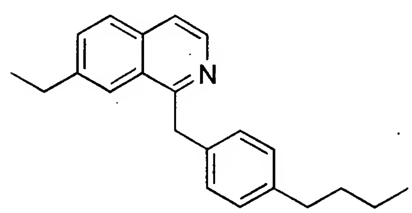
Potassium carbonate (13 mg, 0.094 mmol) was added to a solution of the compound of Example B157 (6 mg, 0.016 mmol) in methanol (1.0 ml), and the mixture was stirred at room temperature for 1 hour. Upon

concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography to give the title compound (3.0 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.91(3H, t), 1.29-1.38(2H, m), 1.52-1.57(2H, m), 2.55(2H, t), 3.19(1H, s), 4.62(2H, s), 7.09(2H, d), 7.20(2H, d), 7.53(1H, d), 7.67-7.69(1H, m), 7.77(1H, d), 8.36(1H, s), 8.52(1H, d)

Example B159

1-(4-Butylbenzyl)-7-ethylisoquinoline

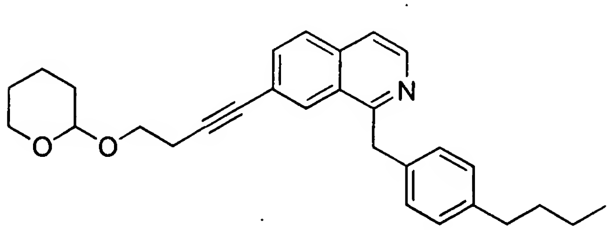


Palladium-carbon (10%, 5.0 mg) was added to a solution of the compound of Example B158 (2.0 mg) in tetrahydrofuran (2.0 ml), and the mixture was stirred at room temperature under nitrogen atmosphere (1 atm) for 1 hour. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography to give the title compound (0.21 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(6H, t), 1.25-1.32(2H, m), 1.48-1.57(2H, m), 2.53(2H, t), 2.80(2H, q), 4.62(2H, s), 7.06(2H, d), 7.20(2H, d), 7.49-7.52(2H, m), 7.73(1H, d), 7.95(1H, s), 8.43(1H, d)

Example B160

1-(4-Butylbenzyl)-7-[4-(tetrahydro-2*H*-2-pyranyloxy)-1-butynyl]-isoquinoline



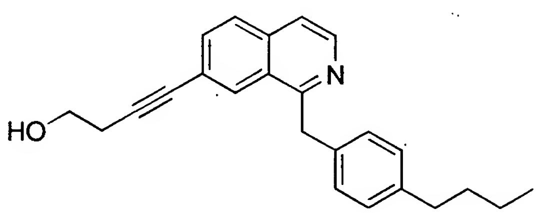
Palladium acetate (11 mg, 0.047 mmol), 1,1'-bis(diphenylphosphino)ferrocene (72 mg, 0.13 mmol), and lithium

chloride (25 mg, 0.59 mmol) were added to a solution of the compound of Example B155 (100 mg, 0.24 mmol) and 2-(3-butynyloxy)tetrahydro-2*H*-pyran (73 mg, 0.47 mmol) in dimethylformamide (3.0 ml), and the system was purged with nitrogen. Furthermore, triethylamine (59 µl, 0.43 mmol) and copper iodide (2 mg, 0.018 mmol) were added, and the resulting mixture w as stirred at 80°C for 24 hours. The mixture was cooled to room temperature, water was added, and the resulting mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (25 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.28-1.38(2H, m), 1.52-1.67(6H, m), 1.72-1.79(1H, m), 1.79-1.88(1H, m), 2.54(2H, t), 2.78(2H, t), 3.53-3.56(1H, m), 3.66-3.72(1H,m), 3.91-3.99(2H, m), 4.60(2H, s), 4.71-4.73(1H, m), 7.08(2H, d), 7.19(2H, d), 7.50(1H, d), 7.59-7.62(1H, m), 7.72(1H, d), 8.24(1H, s), 8.48(1H, d)

Example B161

4-[1-(4-Butylbenzyl)-7-isoquinolyl]-3-butyn-1-ol

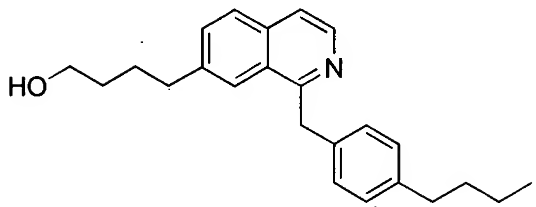


The title compound was obtained by treating the compound of Example B160 in the same manner as in Example B29.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.39(2H, m), 1.51-1.57(2H, m), 1.83(1H, brs), 2.55(2H, t), 2.75(2H, t), 3.84-3.89(2H, m), 4.60(2H, s), 7.08(2H, d), 7.18(2H, d), 7.50(1H, d), 7.60-7.62(1H, m), 7.73(1H, d), 8.25(1H, s), 8.48(1H, d)

Example B162

4-[1-(4-Butylbenzyl)-7-isoquinolyl]-1-butanol

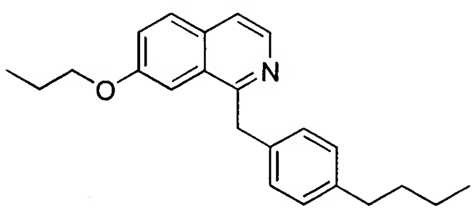


The title compound was obtained by treating the compound of Example B161 in the same manner as in Example B30.

^{1}H-NMR($CDCl_3$) δ (ppm): 0.89(3H, t), 1.28-1.36(2H, m), 1.50-1.59(4H, m), 1.67-1.77(3H, m), 2.53(2H, t), 2.79(2H, t), 3.63(2H, t), 4.62(2H, s), 7.06(2H, d), 7.18(2H, d), 7.47-7.52(2H, m), 7.73(1H, d), 7.92(1H, s), 8.43(1H, d)

Example B163

1-(4-Butylbenzyl)-7-propoxyisoquinoline

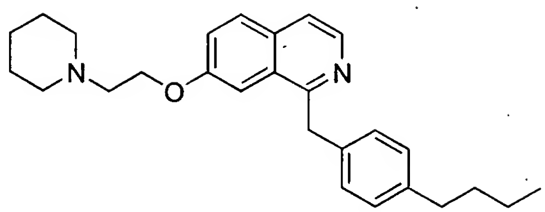


The title compound was obtained by treating the compound of Example B154 in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm): 0.90(3H, t), 1.05(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 1.76-1.84(2H, m), 2.53(2H, t), 3.92(2H, t), 4.58(2H, s), 7.06(2H, d), 7.19(2H, d), 7.26-7.29(1H, m), 7.34(1H, d), 7.48(1H, d), 7.70(1H, d), 8.38(1H, d)

Example B164

1-(4-Butylbenzyl)-7-(2-piperidinoethoxy)isoquinoline

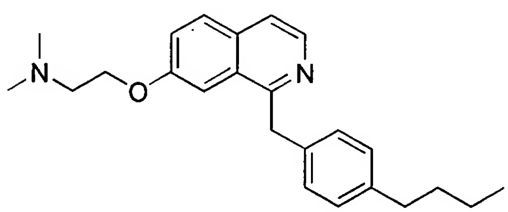


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.43-1.58(4H, m), 1.61-1.69(4H, m), 2.51-2.55(6H, m), 2.79(2H, t), 4.11(2H, t), 4.57(2H, s), 7.06(2H, d), 7.18(2H, d), 7.28-7.30(1H, m), 7.36(1H, d), 7.48(1H, d), 7.70(1H, d), 8.38(1H, d)

Example B165

N-(2-{[1-(4-butylbenzyl)-7-isoquinolyl]oxy}ethyl)-*N*,*N*-dimethyl-amine

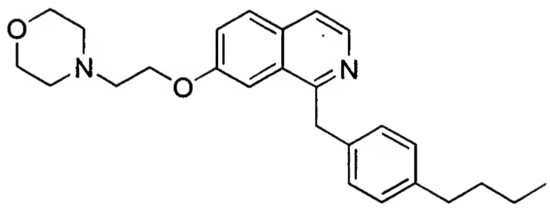


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.57(2H, m), 2.35(6H, s), 2.53(2H, t), 2.75(2H, t), 4.06(2H, t), 4.58(2H, s), 7.06(2H, d), 7.18(2H, d), 7.30-7.33(1H, m), 7.36(1H, d), 7.48(1H, d), 7.70(1H, d), 8.39(1H, d)

Example B166

1-(4-Butylbenzyl)-7-isoquinolyl-(2-morpholinoethyl) ether

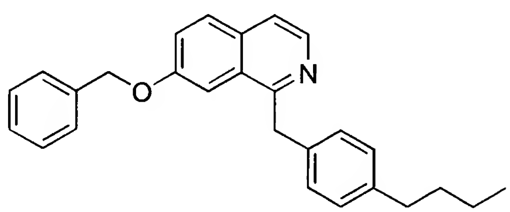


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.51-2.58(6H, m), 2.81(2H, t), 3.75(4H, t), 4.11(2H, t), 4.58(2H, s), 7.06(2H, d), 7.17(2H, d), 7.28-7.31(1H, m), 7.35(1H, d), 7.49(1H, d), 7.71(1H, d), 8.39(1H, d)

Example B167

7-(Benzyloxy)-1-(4-butylbenzyl)isoquinoline

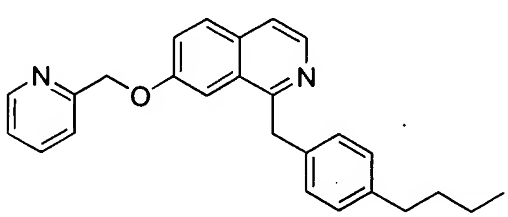


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.54(2H, m), 2.54(2H, t), 4.54(2H, s), 5.06(2H, s), 7.05(2H, d), 7.14(2H, d), 7.34-7.43(7H, m), 7.49(1H, d), 7.72(1H, d), 8.39(1H, d)

Example B168

1-(4-Butylbenzyl)-7-(2-pyridylmethoxy)isoquinoline



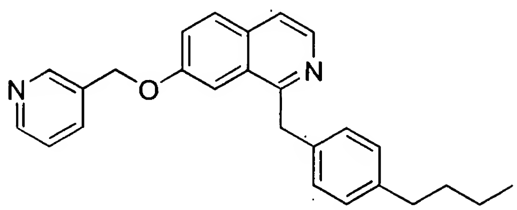
The title compound was obtained in the same manner as in Example

B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.49-1.57(2H, m), 2.52(2H, t), 4.51(2H, s), 5.25(2H, s), 7.02(2H, d), 7.14(2H, d), 7.24-7.27(1H, m), 7.40(1H, dd), 7.47-7.50(3H, m), 7.68-7.72(1H, d), 7.74(1H, d), 8.39(1H, d), 8.64-8.66(1H, m)

Example B169

1-(4-Butylbenzyl)-7-(3-pyridylmethoxy)isoquinoline

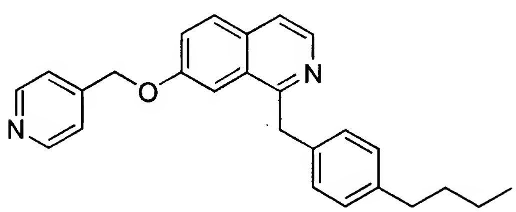


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.54(2H, t), 4.57(2H, s), 5.06(2H, s), 7.07(2H, d), 7.15(2H, d), 7.31-7.36(2H, m), 7.42(1H, d), 7.51(1H, d), 7.74-7.76(2H, m), 8.42(1H, d), 8.61-8.62(1H, m), 8.69-8.70(1H, m)

Example B170

1-(4-Butylbenzyl)-7-(4-pyridylmethoxy)isoquinoline

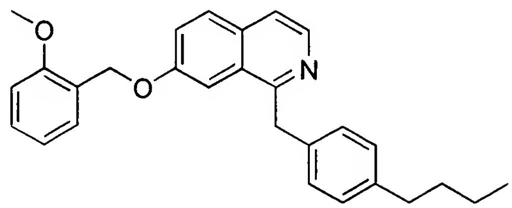


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 2.54(2H, t), 4.53(2H, s), 5.09(2H, s), 7.04(2H, d), 7.09(2H, d), 7.33-7.39(4H, m), 7.51(1H, d), 7.76(1H, d), 8.41(1H, d), 8.63-8.64(2H, m)

Example B171

1-(4-Butylbenzyl)-7-[(2-methoxybenzyl)oxy]isoquinoline

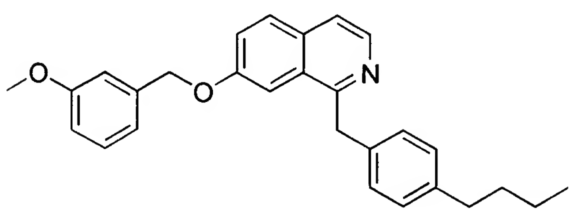


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.57(2H, m), 2.53(2H, t), 3.82(3H, s), 4.52(2H, s), 5.04(2H, s), 6.88-6.91(1H, m), 6.99-7.02(2H, m), 7.05(2H, d), 7.14(2H, d), 7.32(1H, t), 7.36(1H, dd), 7.43(1H, d), 7.48(1H, d), 7.72(1H, d), 8.39(1H, d)

Example B172

1-(4-Butylbenzyl)-7-[(3-methoxybenzyl)oxy]isoquinoline

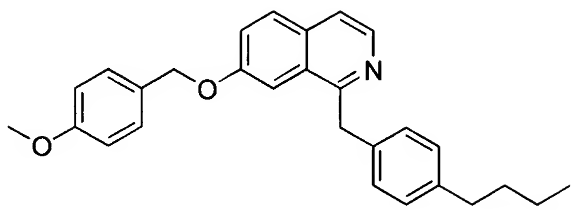


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 2.53(2H, t), 3.90(3H, s), 4.53(2H, s), 5.16(2H, s), 6.93-6.98(2H, m), 7.03(2H, d), 7.15(2H, d), 7.30-7.35(1H, m), 7.37(1H, dd), 7.41-7.43(1H, m), 7.47(1H, d), 7.51(1H, d), 7.71(1H, d), 8.37(1H, d)

Example B173

1-(4-Butylbenzyl)-7-[(4-methoxybenzyl)oxy]isoquinoline

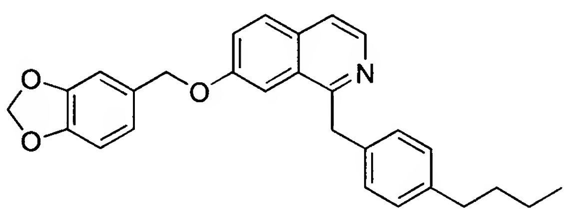


The title compound was obtained in the same manner as in Example B148.

[1]H-NMR$(CDCl_3)$ δ (ppm):0.89(3H, t), 1.27-1.37(2H, m), 1.51-1.57(2H, m), 2.54(2H, t), 3.83(3H, s), 4.55(2H, s), 4.99(2H, s), 6.93(2H, d), 7.06(2H, d), 7.15(2H, d), 7.32-7.36(3H, m), 7.44(1H, d), 7.48(1H, d), 7.71(1H, d), 8.38(1H, d)

Example B174

7-(1,3-Benzodioxol-5-ylmethoxy)-1-(4-butylbenzyl)isoquinoline

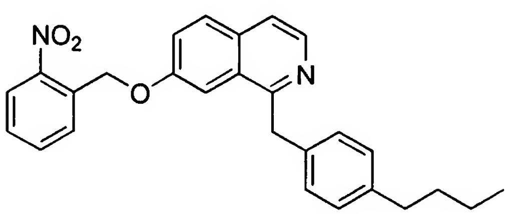


The title compound was obtained in the same manner as in Example B148.

[1]H-NMR$(CDCl_3)$ δ (ppm):0.89(3H, t), 1.27-1.37(2H, m), 1.51-1.57(2H, m), 2.54(2H, t), 4.55(2H, s), 4.95(2H, s), 5.98(2H, s), 6.82(1H, d), 6.88(1H, dd), 6.92(1H, d), 7.06(2H, d), 7.15(2H, d), 7.33(1H, dd), 7.42(1H, d), 7.48(1H, d), 7.72(1H, d), 8.39(1H, d)

Example B175

1-(4-Butylbenzyl)-7-[(2-nitrobenzyl)oxy]isoquinoline

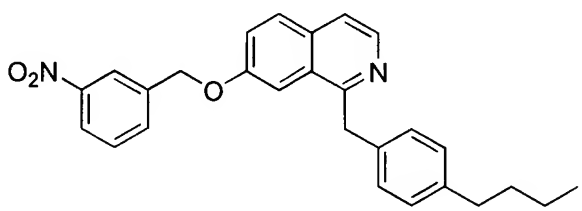


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.87(3H, t), 1.26-1.34(2H, m), 1.48-1.56(2H, m), 2.51(2H, t), 4.53(2H, s), 5.49(2H, s), 7.03(2H, d), 7.14(2H, d), 7.40(1H, dd), 7.430-7.434(1H, m), 7.45-7.49(1H, m), 7.51(1H, d), 7.64-7.68(1H, m), 7.76(1H, d), 7.85-7.87(1H, m), 8.22-8.24(1H, d), 8.41(1H, d)

Example B176

1-(4-Butylbenzyl)-7-[(3-nitrobenzyl)oxy]isoquinoline

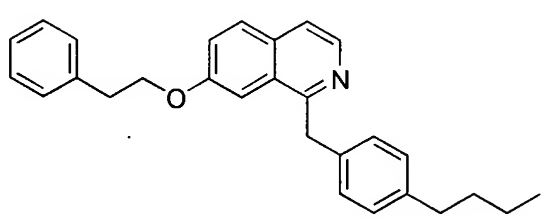


The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.56(2H, m), 2.54(2H, t), 4.55(2H, s), 5.14(2H, s), 7.05(2H, d), 7.11(2H, d), 7.37-7.40(2H, m), 7.51(1H, d), 7.55-7.59(1H, m), 7.73-7.78(2H, m), 8.19-8.22(1H, m), 8.32-8.33(1H, m), 8.42(1H, d)

Example B177

1-(4-Butylbenzyl)-7-(phenethyloxy)isoquinoline



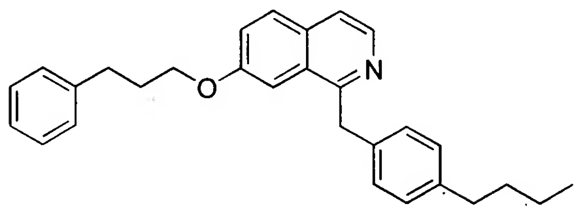
The title compound was obtained in the same manner as in Example B148.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.26-1.36(2H, m), 1.49-1.57(2H, m), 2.52(2H, t), 3.10(2H, t), 4.18(2H, t), 4.56(2H, s), 7.04(2H, d), 7.16(2H, d), 7.26-7.28(4H, m), 7.33-7.35(3H, m), 7.48(1H, d), 7.70(1H, d),

8.38-8.39(1H, m)

Example B178

1-(4-Butylbenzyl)-7-(3-phenylpropoxy)isoquinoline

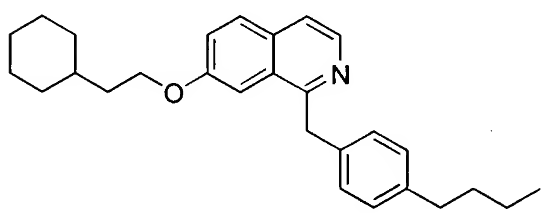


The title compound was obtained in the same manner as in Example B148.

[1]H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.49-1.57(2H, m), 2.09-2.15(2H, m), 2.52(2H, t), 2.82(2H, t), 3.97(2H, t), 4.55(2H, s), 7.04(2H, d), 7.16(2H, d), 7.20-7.23(3H, m), 7.27-7.33(4H, m), 7.48(1H, d), 7.70(1H, d), 8.38(1H, d)

Example B179

1-(4-Butylbenzyl)-7-(2-cyclohexylethoxy)isoquinoline

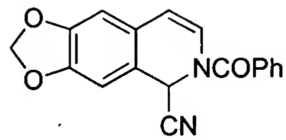


The title compound was obtained in the same manner as in Example B148.

[1]H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 0.94-1.02(2H, m), 1.17-1.36(4H, m), 1.36-1.57(4H, m), 1.65-1.76(7H, m), 2.53(2H, t), 3.98(2H, t), 4.58(2H, s), 7.06(2H, d), 7.19(2H, d), 7.25-7.28(1H, m), 7.33(1H, d), 7.47(1H, d), 7.69(1H, d), 8.37(1H, d)

Example B180

6-Benzoyl-5,6-dihydro[1,3]dioxolo[4,5-*g*]isoquinoline-5-carbonitrile

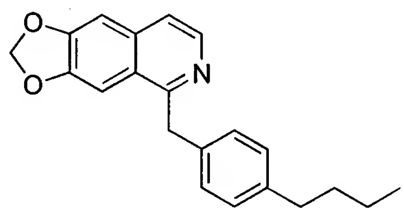


The title compound was obtained by treating [1,3]dioxolo[4,5-*g*]isoquinoline in the same manner as in Example B140.

^{1}H-NMR($CDCl_3$) δ (ppm):5.94-5.96(1H, m), 6.03(1H, d), 6.04(1H, d), 6.47-6.54(2H, m), 6.70(1H, s), 6.83(1H, s), 7.45-7.49(2H, m), 7.54-7.62(3H, m)

Example B181

5-(4-Butylbenzyl)[1,3]dioxolo[4,5-*g*]isoquinoline

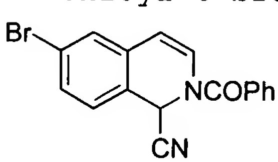


The title compound was obtained by treating the compound of Example B180 and the compound of Example B1 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.57(2H, m), 2.54(2H, t), 4.50(2H, s), 6.05(2H, s), 7.05-7.07(3H, m), 7.16(2H, d), 7.38(7.40(2H, m), 8.35(1H, d)

Example B182

2-Benzoyl-6-bromo-1,2-dihydro-1-isoquinolinecarbonitrile

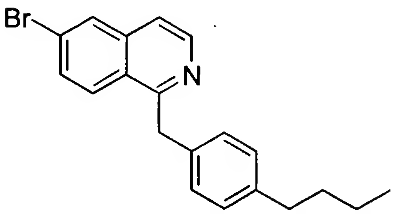


The title compound was obtained by treating 6-bromoisoquinoline, which was synthesized according to J. Am. Chem. Soc., 183 (1942), in the same manner as in Example B140.

^{1}H-NMR($CDCl_3$) δ (ppm):6.01(1H, d), 6.53(1H, brs), 6.70(1H, brd), 7.24(1H, d), 7.33(1H, d), 7.47-7.51(3H, m), 7.56(3H, m)

Example B183

6-Bromo-1-(4-butylbenzyl)isoquinoline

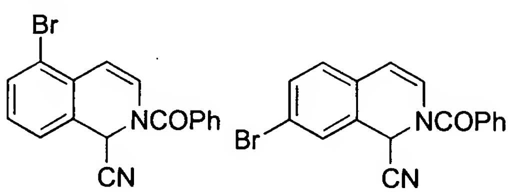


The title compound was obtained by treating the compound of Example B182 and the compound of Example B1 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.89(3H, t), 1.27-1.36(2H, m), 1.50-1.58(2H, m), 2.53(2H, t), 4.60(2H, s), 7.06(2H, d), 7.15(2H, d), 7.46(1H, d), 7.59(1H, q), 7.98(1H, d), 8.02(1H, d), 8.51(1H, d)

Example B184

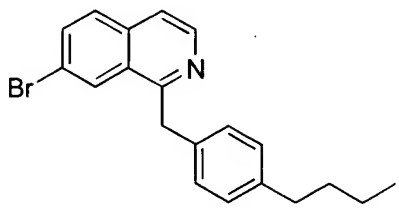
A mixture of 2-benzoyl-5-bromo-1,2-dihydro-1-isoquinoline-carbonitrile and 2-benzoyl-7-bromo-1,2-dihydro-1-isoquinoline-carbonitrile



The title compounds were obtained by treating 5- or 7-bromoisoquinoline, which was synthesized according to J. Am. Chem. Soc., 61, 183 (1939), in the same manner as in Example B140. The obtained compounds were used in the following reaction without separation and purification.

Example B185

7-Bromo-1-(4-butylbenzyl)isoquinoline

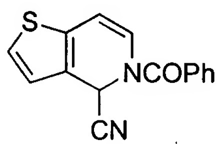


The title compound was obtained by treating the compound of Example B184 and the compound of Example B1 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.58(2H, m), 2.55(2H, t), 4.58(2H, s), 7.09(2H, d), 7.18(2H, d), 7.51-7.53(1H, m), 7.69-7.70(2H, m), 8.33-8.34(1H, m), 8.52(1H, d)

Example B186

5-Benzoyl-4,5-dihydrothieno[3,2-*c*]pyridine-4-carbonitrile

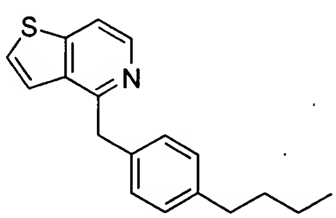


The title compound was obtained by treating thieno[3,2-*c*]pyridine, synthesized according to J. Heterocycl. Chem., 30, 183 (1993), in the same manner as in Example B140.

^{1}H-NMR($CDCl_3$) δ (ppm):6.05(1H, d), 6.57(1H, brd), 6.66(1H, s), 7.07(1H, d), 7.32(1H, d), 7.46-7.50(2H, m), 7.54-7.62(3H, m)

Example B187

4-(4-Butylbenzyl)thieno[3,2-*c*]pyridine

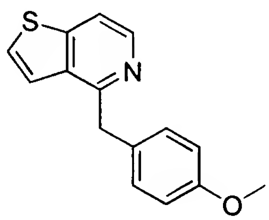


The title compound was obtained by treating the compound of Example B186 and the compound of Example B1 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.27-1.37(2H, m), 1.51-1.59(2H, m), 2.54(2H, t), 4.47(2H, s), 7.07(2H, d), 7.19(2H, d), 7.42(1H, d), 7.47(1H, dd), 7.68(1H, d), 8.41(1H, d)

Example B188

4-(4-Methoxybenzyl)thieno[3,2-*c*]pyridine

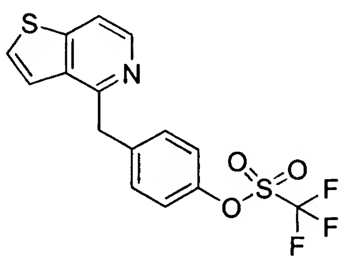


The title compound was obtained by treating the compound of Example B186 and 4-methoxybenzyl chloride in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):3.75(3H, s), 4.44(2H, s), 6.79-6.82(2H, m), 7.19-7.22(2H, m), 7.43(1H, d), 7.46(1H, dd), 7.68(1H, d), 8.41(1H, d)

Example B189

4-(Thieno[3,2-*c*]pyridin-4-ylmethyl)phenyl trifluoromethane-sulfonate



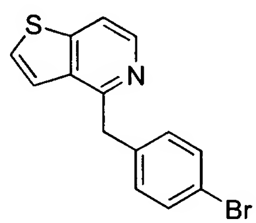
A solution of boron tribromide in methylene chloride (1.0 M, 10 ml, 10 mmol) was added dropwise to a solution of the compound of Example B188 (510 mg, 2.0 mmol) in methylene chloride (10 ml)cooled to 0°C , and this reaction mixture was stirred at that temperature for 1.5 hours. The reaction mixture was made weakly alkaline by addition of a saturated aqueous sodium hydrogencarbonate solution, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The obtained residue was dissolved in pyridine, and

the resulting solution was cooled to 0°C. After trifluoromethanesulfonic anhydride (0.34 ml, 2.1 mmol) was added dropwise thereto, the mixture was stirred at that temperature for 2 hours, poured on ice, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography to give the title compound (312 mg).

^{1}H-NMR($CDCl_3$) δ (ppm):4.52(2H, s), 7.16-7.18(2H, m), 7.36(2H, m), 7.43-7.44(1H, m), 7.49(1H, d), 7.73(1H, d), 8.42(1H, d)

Example B190

4-(4-Bromobenzyl)thieno[3,2-*c*]pyridine

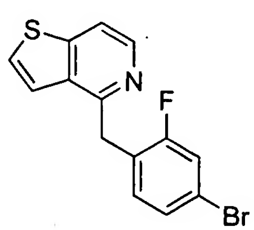


The title compound was obtained by treating the compound of Example B186 and the compound of Example B31 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.45(2H, s), 7.14-7.16(2H, m), 7.37-7.39(2H, m), 7.41-7.43(1H, m), 7.45(1H, d), 7.71(1H, d), 8.41(1H, d)

Example B191

4-(4-Bromo-2-fluorobenzyl)thieno[3,2-*c*]pyridine

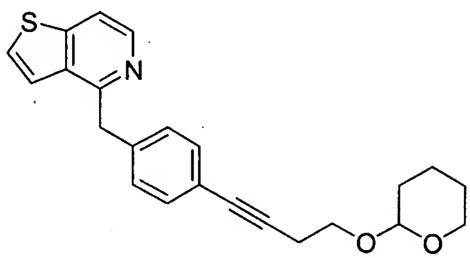


The title compound was obtained by treating the compound of Example B186 and 4-bromo-2-fluorobenzyl bromide in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.46(2H, s), 7.11(1H, t), 7.15-7.18(1H, m), 7.22-7.25(1H, m), 7.47(1H, d), 7.49(1H, d), 7.71(1H, d), 8.41(1H, d)

Example B192

4-{4-[4-(Tetrahydro-2*H*-2-pyranyloxy)-1-butynyl]benzyl}thieno[3,2-*c*]pyridine

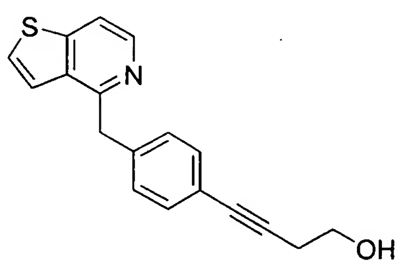


The title compound was obtained by treating the compound of Example B189 and 2-(3-butynyloxy)tetrahydro-2*H*-pyran in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.40-1.90(6H, m), 2.69(2H, t), 3.45-3.65(2H, m), 3.78-3.95(2H, m), 4.48(2H, s), 4.66-4.69(1H, m), 7.18(2H, d), 7.27(2H, d), 7.41(1H, d), 7.44(1H, d), 7.70(1H, d), 8.41(1H, d).

Example B193

4-[4-(Thieno[3,2-*c*]pyridin-4-ylmethyl)phenyl]-3-butyn-1-ol



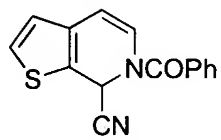
The title compound was obtained by treating the compound of Example B192 in the same manner as in Example B47.

^{1}H-NMR($CDCl_3$) δ (ppm):2.67(2H, t), 3.79(2H, t), 4.50(2H, s), 7.20(2H, d), 7.32(2H, d), 7.41(1H, d), 7.44(1H, d), 7.71(1H, d), 8.42(1H, d).

The proton of the hydroxyl group was not observed in the NMR spectrum.

Example B194

6-Benzoyl-6,7-dihydrothieno[2,3-*c*]pyridine-7-carbonitrile

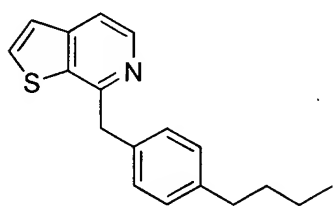


The title compound was obtained by treating thieno[2,3-*c*]pyridine, which was synthesized according to J. Heterocycl. Chem., 30, 183 (1993), in the same manner as in Example B140.

^{1}H-NMR($CDCl_3$) δ (ppm):6.07(1H, d), 6.56(1H, brd), 6.75(1H, s), 6.97(1H, d), 7.37(1H, d), 7.46-7.51(2H, m), 7.54-7.64(3H, m)

Example B195

7-(4-Butylbenzyl)thieno[2,3-*c*]pyridine

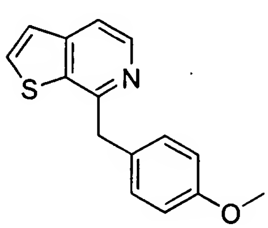


The title compound was obtained by treating the compound of Example B194 and the compound of Example B1 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.59(2H, m), 2.55(2H, t), 4.40(2H, s), 7.09(2H, d), 7.28(2H, d), 7.34(1H, d), 7.57(1H, d), 7.62(1H, d), 8.47(1H, d)

Example B196

7-(4-Methoxybenzyl)thieno[2,3-*c*]pyridine

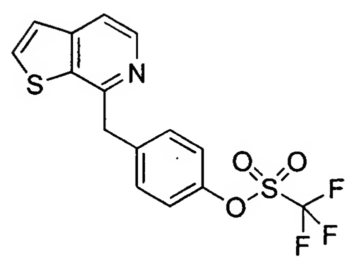


The title compound was obtained by treating the compound of Example B194 and 4-methoxybenzyl chloride in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):3.76(3H, s), 4.38(2H, s), 6.81-6.83(2H, m), 7.28-7.30(2H, m), 7.35(1H, d), 7.57(1H, d), 7.62(1H, d), 8.47(1H, d)

Example B197

4-(Thieno[2,3-*c*]pyridin-7-ylmethyl)phenyl trifluoromethane-sulfonate

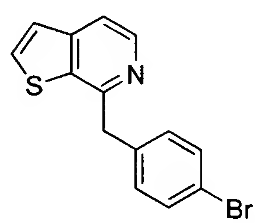


The title compound was obtained by treating the compound of Example B196 in the same manner as in Example B189.

^{1}H-NMR($CDCl_3$) δ (ppm):4.44(2H, s), 7.17-7.19(2H, m), 7.38-7.40(1H, m), 7.44-7.46(2H, m), 7.61(1H, d), 7.65-7.67(1H, m), 8.47-8.49(1H, m)

Example B198

7-(4-Bromobenzyl)thieno[2,3-*c*]pyridine

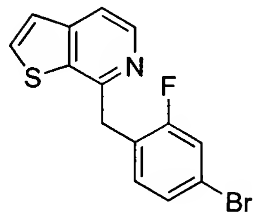


The title compound was obtained by treating the compound of Example B194 and the compound of Example B31 in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.37(2H, s), 7.23-7.25(2H, m), 7.37(1H, d), 7.39-7.41(2H, m), 7.59(1H, d), 7.63-7.65(1H, m), 8.47(1H, d)

Example B199

7-(4-Bromo-2-fluorobenzyl)thieno[2,3-*c*]pyridine

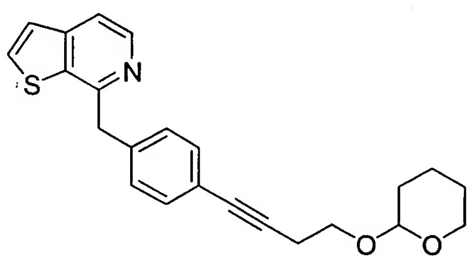


The title compound was obtained by treating the compound of Example B194 and 4-bromo-2-fluorobenzyl bromide in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.40-4.41(2H, m), 7.12-7.20(2H, m), 7.23-7.26(1H, m), 7.37-7.39(1H, m), 7.59-7.62(1H, m), 7.65-7.67(1H, m), 8.45-8.47(1H, m)

Example B200

7-{4-[4-(Tetrahydro-2*H*-2-pyranyloxy)-1-butynyl]benzyl}thieno[2,3-*c*]pyridine

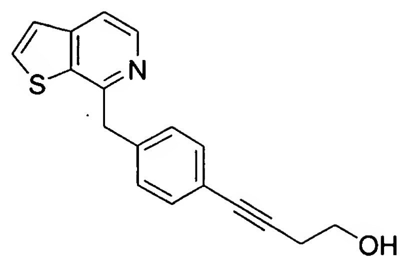


The title compound was obtained by treating the compound of Example B197 and 2-(3-butynyloxy)tetrahydro-2*H*-pyran in the same manner as in Example B42.

^{1}H-NMR($CDCl_3$) δ (ppm):1.50-1.90(6H, m), 2.69(2H, t), 3.49-3.54(1H, m), 3.58-3.65(1H, m), 3.85-3.95(2H, m), 4.41(2H, s), 4.68(1H, t), 7.26-7.31(4H, m), 7.36(1H, d), 7.58(1H, d), 7.63(1H, d), 8.47(1H, d).

Example B201

4-[4-(Thieno[2,3-*c*]pyridin-7-ylmethyl)phenyl]-3-butyn-1-ol

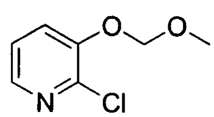


The title compound was obtained by treating the compound of Example B200 in the same manner as in Example B47.

^{1}H-NMR($CDCl_3$) δ (ppm): 1.99(1H, brs), 2.67(2H, t), 3.79(2H, t), 4.42(2H, s), 7.27-7.34(4H, m), 7.36(1H, d), 7.59(1H, d), 7.64(1H, d), 8.47(1H, d).

Example B202

2-Chloro-3-(methoxymethoxy)pyridine

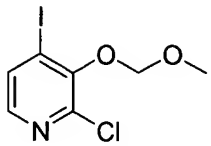


Sodium hydride (66%, 633 mg, 17.4 mmol) was added to an ice-cooled solution of 2-chloro-3-hydroxypyridine (2.05 g, 15.8 mmol) in tetrahydrofuran (30 ml) under nitrogen atmosphere, and this reaction mixture was stirred at that temperature for 15 minutes. Chloromethyl methyl ether (1.32 ml, 17.4 mmol) was added, and the resulting reaction mixture was stirred at that temperature for 30 minutes, then at room temperature for another 2 hours. After water was added, the reaction mixture was extracted with ethyl acetate, washed with saturated brine, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (2.44 g).

^{1}H-NMR($CDCl_3$) δ (ppm): 3.53(3H, s), 5.28(2H, s), 7.19(1H, dd), 7.49(1H, dd), 8.06(1H, dd)

Example B203

2-Chloro-4-iodo-3-(methoxymethoxy)pyridine

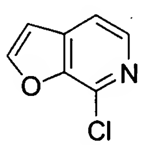


A solution of the compound of Example B202 (1.40 g, 8.06 mmol) in diethyl ether (8 ml) was added dropwise to a solution of 1.51 M *t*-butyllithium-*n*-pentane solution (8.01 ml, 12.1 mmol) in diethyl ether (15 ml) cooled to -78°C under nitrogen atmosphere, and the reaction mixture was stirred at that temperature for 15 minutes. After iodine (3.07 g, 12.1 mmol) was added, the reaction mixture was gradually warmed to room temperature. An aqueous sodium thiosulfate solution was further added, and the diethyl ether layer was separated, washed with saturated brine, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (356 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 3.73(3H, s), 5.22(2H, s), 7.69(1H, d), 7.80(1H, d)

Example B204

7-Chlorofuro[2,3-*c*]pyridine



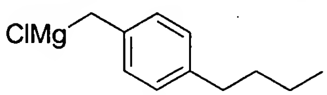
Trimethylsilylacetylene (28.3 µl, 0.201 mmol) and triethylamine (59.8 µl, 0.429 mmol) were added to a solution of the compound of Example B203 (36.6 mg, 0.143 mmol), tetrakis(triphenylphosphine)palladium (16.5 mg, 0.0143 mmol), and copper(I) iodide (2.7 mg, 0.014 mmol) in dimethylformamide (1.5 ml) , and this mixture was stirred at 50°C for 4 hours. After allowing the mixture to cool to room temperature, water was added thereto, and the resulting mixture was extracted with ethyl acetate, washed with saturated brine, and then concentrated under reduced pressure. The residue was dissolved in methanol (5 ml), potassium carbonate (100 mg, 0.724 mmol) was added thereto, and the resulting mixture was stirred at room temperature for 1 hour. After

water was added, the mixture was extracted with diethyl ether, washed with saturated brine, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (5.5 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 6.89(1H, d), 7.51(1H, d), 7.83(1H, d), 8.21(1H, d).

Example B205

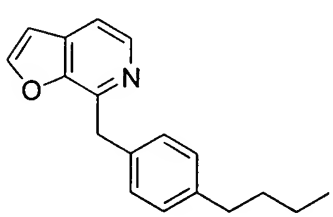
4-Butylbenzylmagnesium chloride



A mixed solution of the compound of Example B1 (1.04 g, 5.69 mmol), magnesium (761 mg, 31.3 mmol), and a catalytic amount of 1,2-dibromoethane in diethyl ether (11 ml) was initiated by heating under reflux. After the heat source was removed, a solution of the compound of Example B1 (4.16 g, 22.8 mmol) in diethyl ether (60 ml) was added dropwise to the reaction mixture at a rate that maintains gentle reflux, and the mixture was heated under reflux for 30 minutes. The mixture was then allowed to cool to room temperature to give the title compound as a 0.4 M solution in diethyl ether. This solution was used in the following reaction as it is.

Example B206

7-(4-Butylbenzyl)furo[2,3-*c*]pyridine



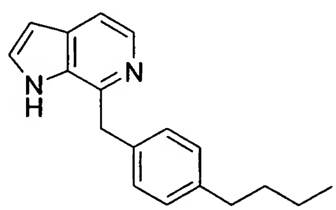
The compound of Example B205 (300 µl, 0.1 mmol) was added to a solution of the compound of Example B204 (5.0 mg, 0.033 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloronickel(II) (4.5 mg, 0.0065 mmol) in tetrahydrofuran (1 ml), and the mixture was stirred at 50°C for 1 hour. After allowing the mixture to cool to room temperature,

ethyl acetate was added thereto. The resulting mixture was washed with a saturated aqueous ammonium chloride solution and saturated brine, then concentrated under reduced pressure. The residue was purified by NH-silica gel column chromatography to give the title compound (2.9 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.89(3H, t), 1.29-1.35(2H, m), 1.50-1.58(2H, m), 2.54(2H, t), 4.40(2H, s), 6.78(1H, d), 7.08(2H, d), 7.30(2H, d), 7.40(1H, d), 7.72(1H, d), 8.34(1H, d)

Example B207

7-(4-Butylbenzyl)-1*H*-pyrrolo[2,3-*c*]pyridine



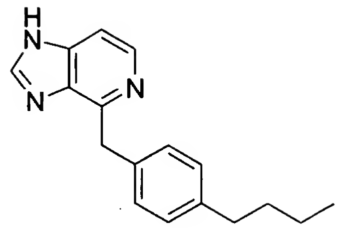
The compound of Example B205 (800 µl, 0.3 mmol) was added to a solution of 1-chloropyrrolopyridine (19.4 mg, 0.127 mmol), which was synthesized from 2-chloro-3-aminopyridine according to the method of H07-165,708A, and dichloro(diphenylphosphinopropane)nickel (6.9 mg, 0.013 mmol) in tetrahydrofuran (1 ml) under ice-cooling, and the mixture was stirred while heating under reflux for 4 hours. After allowing the mixture to cool to room temperature, ethyl acetate was added thereto. The resulting mixture was washed with a saturated aqueous ammonium chloride solution and saturated brine, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (7.1 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.91(3H, t), 1.31-1.37(2H, m), 1.55-1.59(2H, m), 2.58(2H, t), 4.44(2H, s), 6.50(1H, d), 7.12(2H, d), 7.18(1H, d), 7.22(2H, d), 7.45(1H, d), 8.21(1H, d)

The NH proton was not observed in the NMR spectrum.

Example B208

4-(4-Butylbenzyl)-1-imidazo[4,5-*c*]pyridine



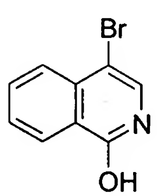
The compound of Example B205 (3.45 ml, 1.38 mmol) was added to a solution of 1-chloroimidazopyridine (88.6 mg, 0.577 mmol), which was synthesized from 4-amino-2-chloropyridine according to the method described in J. Heterocycl. Chem., 2, 196 (1965), and dichloro(diphenylphosphinopropane)nickel (31.3 mg, 0.0577 mmol) in tetrahydrofuran (2 ml), and the mixture was stirred while heating under reflux for 2 hours. After allowing the mixture to cool to room temperature, ethyl acetate was added thereto. The resulting mixture was filtered through silica gel and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (64.2 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.86(3H, t), 1.23-1.32(2H, m), 1.44-1.52(2H, m), 2.47(2H, t), 4.56(2H, s), 7.02(2H, d), 7.19(2H, d), 7.34(1H, d), 8.00(1H, s), 8.25-8.27(1H, m)

The NH proton was not observed in the NMR spectrum.

Example B209

4-Bromo-1-isoquinolinol



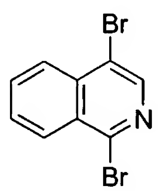
Bromine (1.78 ml, 34.5 mmol) was added to an ice-cooled solution of 1-hydroxyisoquinoline (5.01 g, 34.5 mmol) in acetic acid (50 ml), and this reaction mixture was stirred at room temperature for 2 hours. Water, ethyl acetate, and tetrahydrofuran were added, and the resulting reaction mixture was filtered through filter paper. The organic layer was washed with saturated brine and concentrated under reduced pressure.

The residue was recrystallized from ethyl acetate and hexane to give the title compound (6.19 g).

^{1}H-NMR(DMSO-d6) δ(ppm): 7.56(1H, s), 7.59-7.63(1H, m), 7.76-7.78(1H, m), 7.84-7.89(1H, m), 8.23-8.26(1H, m), 11.59(1H, br s)

Example B210

1,4-Dibromoisoquinoline

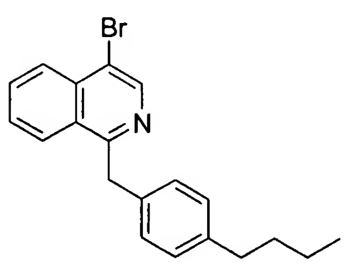


A mixed solution of the compound of Example B209 (1.40 g, 8.06 mmol) and phosphorus tribromide (6 ml) was stirred at 150°C for 1 hour, and then heated under reflux for another 1 hour. The reaction mixture was allowed to cool to room temperature, poured on ice, then warmed to room temperature. Ethyl acetate was added, and the resulting mixture was washed with saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (845 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 7.76-7.80(1H, m), 7.86-7.90(1H, m), 8.19(1H, d), 8.31-8.34(1H, m), 8.48(1H, s)

Example B211

4-Bromo-1-(4-butylbenzyl)isoquinoline



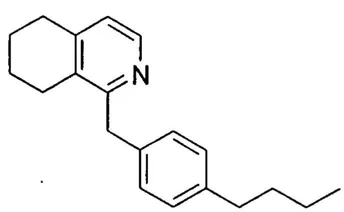
The compound of Example B205 (2.5 ml, 1 mmol) was added to a solution of the compound of Example B210 (200 mg, 0.697 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloronickel(II) (75.6 mg,

0.139 mmol) in tetrahydrofuran (2 ml), and the mixture was stirred at room temperature for 30 minutes. After ethyl acetate was added, the resulting mixture was washed successively with a saturated aqueous ammonium chloride solution, a saturated aqueous sodium hydrogencarbonate solution, and saturated brine, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (98 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.89(3H, t), 1.29-1.34(2H, m), 1.51-1.60(2H, m), 2.53(2H, t), 4.59(2H, s), 7.06(2H, d), 7.16(2H, d), 7.57-7.61(1H, m), 7.73-7.77(1H, m), 8.15-8.19(2H, m), 8.69(1H, s)

Example B212

1-(4-Butylbenzyl)-5,6,7,8-tetrahydroisoquinoline

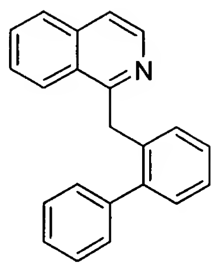


The compound of Example B211 (13.0 mg, 0.0367 mmol) was dissolved in a mixed solution of ethyl acetate and methanol (1:1, 1 ml), 10% palladium-carbon (containing 50% water, 13 mg) was added, and the mixture was stirred at room temperature under hydrogen atmosphere at atmospheric pressure for 12 hours. After purging the reaction system with nitrogen, the catalyst was removed by filtration through celite. The obtained filtrate was concentrated under reduced pressure to give the title compound (8.8 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.90(3H, t), 1.28-1.38(2H, m), 1.52-1.59(2H, m), 1.74-1.82(4H, m), 2.55(2H, t), 2.66(2H, t), 2.81(2H, t), 4.26(2H, s), 7.07-7.15(5H, m), 8.32(1H, d)

Example B213

1-[2-(Phenyl)benzyl]isoquinoline

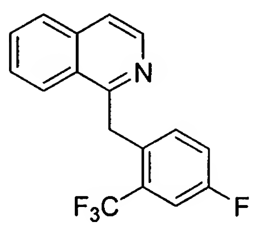


The title compound was obtained by treating 2-phenylbenzyl bromide instead of *n*-butylbenzyl chloride in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.62(2H, s), 7.05(1H, d), 7.16(1H, dd), 7.22-7.50(8H, m), 7.52(1H, d), 7.58(1H, dd), 7.65(1H, d), 7.76(1H, d), 8.47(1H, d).

Example B214

1-[4-Fluoro-2-(trifluoromethyl)benzyl]isoquinoline

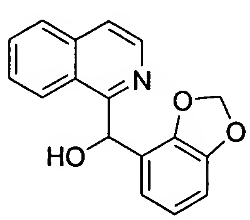


The title compound was obtained by treating 4-fluoro-2-(trifluoromethyl)benzyl methanesulfonate instead of *n*-butylbenzyl chloride in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):4.83(2H, s), 6.87(1H, dd), 7.01(1H, ddd), 7.43(1H, dd), 7.54(1H, dd), 7.61(1H, d), 7.67(1H, dd), 7.85(1H, d), 7.96(1H, d), 8.49(1H, d).

Example B215

1,3-Benzodioxoyl-4-yl-(1-isoquinolyl)methanol

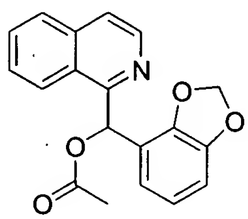


The title compound was obtained by treating2,3-methylenedioxybenzaldehyde in the same manner as in Example B82.

^{1}H-NMR($CDCl_3$) δ (ppm):5.97-5.99(1H, m), 6.09(1H, brs), 6.20-6.40(1H, m), 6.54-6.60(2H, m), 6.65-6.70(2H, m), 7.52(1H, dd), 7.63(1H, d), 7.64(1H, dd), 7.84(1H, d), 8.04(1H, d), 8.53(1H, d).

Example B216

1,3-Benzodioxoyl-4-yl-(1-isoquinolyl)methyl acetate

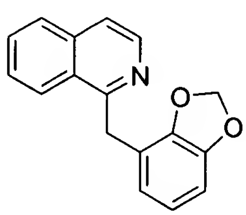


The title compound was obtained by treating the compound of Example B215 in the same manner as in Example B38.

^{1}H-NMR($CDCl_3$) δ (ppm):2.23(3H, s), 5.98-6.02(2H, m), 6.74-6.79(1H, m), 6.90-6.93(1H, m), 7.15-7.19(1H, m), 7.23-7.28(1H, m), 7.58(1H, dd), 7.60(1H, d), 7.66(1H, dd), 7.83(1H, d), 8.28(1H, d), 8.57(1H, d).

Example B217

1-(1,3-Benzodioxoyl-4-ylmethyl)isoquinoline

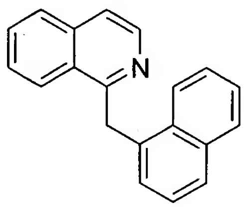


The title compound was obtained by treating the compound of Example B216 in the same manner as in Example B39.

^{1}H-NMR($CDCl_3$) δ (ppm):4.62(2H, s), 6.02(2H, s), 6.64-6.70(3H, m), 7.57(1H, dd), 7.58(1H, d), 7.66(1H, dd), 7.83(1H, d), 8.23(1H, d), 8.50(1H, d).

Example B218

1-(1-Naphthylmethyl)isoquinoline

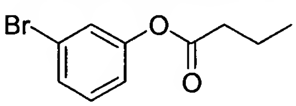


The title compound was obtained by treating 1-(chloromethyl)naphthalene instead of *n*-butylbenzyl chloride in the same manner as in Example B2.

^{1}H-NMR($CDCl_3$) δ (ppm):5.13(2H, s), 6.96(1H, d), 7.29(1H, d), 7.45-7.67(5H, m), 7.72(1H, d), 7.84-7.90(2H, m), 8.08(1H, d), 8.26(1H, d), 8.52(1H, d).

Example B219

3-Bromophenylbutyrate

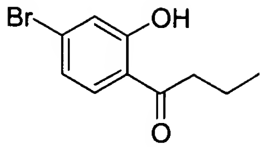


n-Butyryl chloride (7.25 ml) was added to an ice-cooled solution of 3-bromophenol (10.0 g) in pyridine (50 ml), and this reaction mixture was stirred at that temperature for 3 hours, then at room temperature for another 3.5 hours. After ice was added, the reaction mixture was extracted with ethyl acetate, washed with 1 N hydrochloric acid and water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (12.77 g).

^{1}H-NMR(CDC13) δ (ppm): 1.04(3H, t), 1.72-1.82(2H, m), 2.54(2H, t), 7.04(1H, dd), 7.22-7.29(2H, m), 7.36(1H, d).

Example B220

1-(4-Bromo-2-hydroxyphenyl)-1-butanone

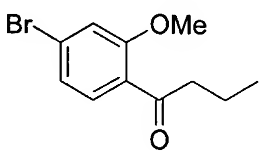


Aluminum chloride (10.51 g) was added to a solution of the compound of Example B219 (12.77 g) in chlorobenzene (70 ml) under nitrogen atmosphere, and this reaction mixture was stirred while heating under reflux for 9 hours. After the reaction mixture was cooled to room temperature, ice was added thereto. The resulting mixture was extracted with ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The compound thus obtained was used in the following reaction without further purification.

^{1}H-NMR(CDCl3) δ (ppm): 0.91(3H, t), 1.53-1.65(2H, m), 3.00(2H, t), 7.02(1H, dd), 7.19(1H, d), 7.78(1H, d), 12.50(1H, s).

Example B221

1-(4-Bromo-2-methoxyphenyl)-1-butanone

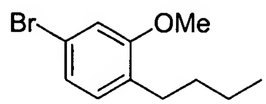


Potassium carbonate (9.07 g) and methyl iodide (3.92 ml) were added to a solution of the compound of Example B220 (13.30 g) in acetone (75 ml), and this reaction mixture was stirred while heating under reflux for 4 hours. The reaction mixture was filtered through celite, ether was added to remove insoluble material by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (9.52 g).

^{1}H-NMR(CDCl3) δ (ppm): 0.95(3H, t), 1.64-1.74(2H, m), 2.91(2H, t), 3.90(3H, s), 7.10(1H, d), 7.14(1H, dd), 7.54(1H, d).

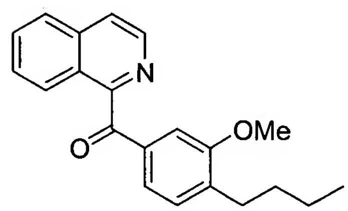
Example B222

4-Bromo-1-butyl-2-methoxybenzene



The title compound was obtained by treating the compound of Example B221 in the same manner as in Example B3.
^{1}H-NMR(CDCl3) δ (ppm): 0.92(3H, t),1.29-1.39(2H, m), 1.48-1.56(2H, m), 2.54(2H, t), 3.81(3H, s), 6.95(1H, s), 6.96-7.02(2H, m).

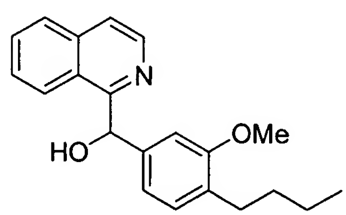
Example B223
(4-Butyl-3-methoxyphenyl) (1-isoquinolyl) ketone



A mixture containing the title compound was obtained by treating the compound of Example B222 in the same manner as in Example B36.

This mixture was used in the following reaction without separation and purification.

Example B224
(4-Butyl-3-methoxyphenyl)(1-isoquinolyl)methanol

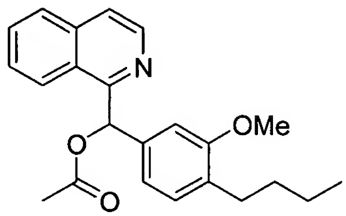


A mixture containing the title compound was obtained by treating the compound of Example B223 in the same manner as in Example B37.

This mixture was used in the following reaction without separation and purification.

Example B225

(4-Butyl-3-methoxyphenyl)(1-isoquinolyl)methyl acetate

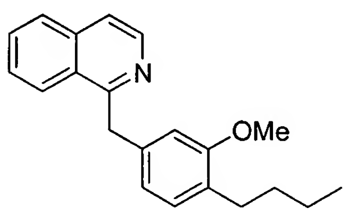


The title compound was obtained by treating the compound of Example B224 in the same manner as in Example B38.

^{1}H-NMR(CDCl3) δ (ppm): 0.90(3H, t), 1.24-1.38(2H, m), 1.46-1.60(2H, m), 2.24(3H, s), 2.54(2H, t), 3.76(3H, s), 6.97(1H, s), 6.98(1H, d), 7.06(1H, d), 7.53-7.67(4H, m), 7.83(1H, d), 8.26(1H, d), 8.58(1H, d).

Example B226

1-(4-Butyl-3-methoxybenzyl)isoquinoline

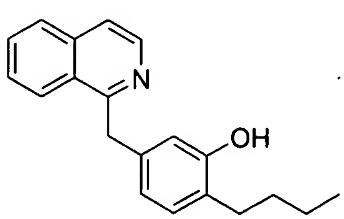


The title compound was obtained by treating the compound of Example B225 in the same manner as in Example B39.

^{1}H-NMR(CDCl3) δ (ppm): 0.89(3H, t), 1.27-1.38(2H, t), 1.45-1.54(2H, t), 2.52(2H, t), 3.72(3H, s), 4.63(2H, s), 6.78(1H, d), 6.79(1H, s), 6.99(1H, d), 7.53(1H, dd), 7.55(1H, d), 7.64(1H, dd), 7.80(1H, d), 8.19(1H, d), 8.49(1H, d).

Example B227

2-Butyl-5-(1-isoquinolylomethyl)phenol



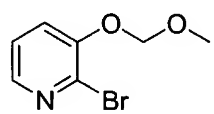
The title compound was obtained by treating the compound of Example B226 in the same manner as in Example B40.

^{1}H-NMR(CDCl3) δ (ppm): 0.91(3H, t), 1.30-1.40(2H, m), 1.52-1.65(2H, m), 2.55(2H, t), 4.55(2H, s), 6.46(1H, brs), 6.85(1H, d), 7.03(1H, d), 7.32-7.40(1H, m), 7.55(1H, dd), 7.68(1H, dd), 7.81(1H, d), 7.94-8.05(1H, m), 8.14(1H, d).

The proton of the phenolic hydroxyl group was not observed in the NMR spectrum.

Example B228

2-Bromo-3-(methoxymethoxy)pyridine

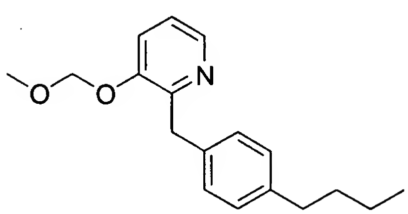


The title compound was synthesized in the same manner as in Example B202 by using 2-bromo-3-hydroxypyridine.

^{1}H-NMR($CDCl_3$) δ (ppm): 3.53(3H, s), 5.29(2H, s), 7.19-7.23(1H, m), 7.42-7.45(1H, m), 8.04-8.06(1H, m)

Example B229

2-(4-Butylbenzyl)-3-(methoxymethoxy)pyridine



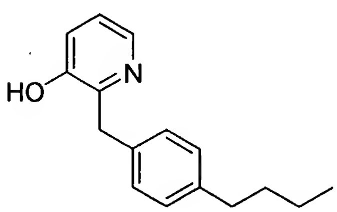
The compound of Example B205 (7 ml, 3 mmol) was added to an ice-cooled mixed solution of the compound of Example B228 (524 mg, 2.40 mmol) and dichloro(diphenylphosphinopropane)nickel (65.0 mg, 0.120 mmol) in tetrahydrofuran (10 ml), and the mixture was stirred while heating under reflux for 5 hours. After allowing the mixture to cool to room temperature, ethyl acetate was added. The resulting mixture was washed successively with a saturated aqueous ammonium chloride

solution, a saturated aqueous sodium hydrogencarbonate solution, and saturated brine, then concentrated under reduced pressure. The residue was filtered through NH-silica gel. After concentrating under reduced pressure, the residue was dissolved in methanol (15 ml), triethylamine (500 μl, 3.59 mmol) and 10% palladium-carbon (containing 50% water, 50 mg) were added, and the resulting mixture was stirred at room temperature under hydrogen atmosphere at atmospheric pressure for 3 hours. After purging the reaction system with nitrogen, the catalyst was removed by filtration through celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (280 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.89(3H, t), 1.28-1.34(2H, m), 1.52-1.58(2H, m), 2.53(2H, t), 3.33(3H, s), 4.16(2H, s), 5.16(2H, s), 7.04-7.10(3H, m), 7.20(2H, d), 7.33-7.35(1H, m), 8.19-8.20(1H, m)

Example B230

2-(4-Butylbenzyl)-3-pyridinol



Trifluoroacetic acid (1 ml) was added to a solution of the compound of Example B229 (256 mg, 0.849 mmol) in methylene chloride (5 ml), and this reaction mixture was stirred at room temperature overnight. After a saturated aqueous sodium hydrogencarbonate solution and ethyl acetate were added, the reaction mixture was washed with saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (182 mg).

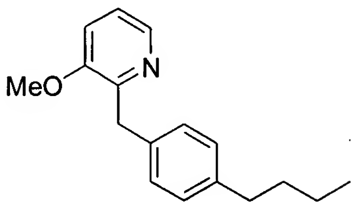
^{1}H-NMR($CDCl_3$) δ (ppm): 0.90(3H, t), 1.28-1.37(2H, m), 1.51-1.58(2H, m), 2.54(2H, t), 4.20(2H, s), 7.02-7.08(4H, m), 7.22(2H, d), 8.08-8.09(1H, m)

The proton of the phenolic hydroxyl group was not observed in the

NMR spectrum.

Example B231

2-(4-Butylbenzyl)-3-methoxypyridine

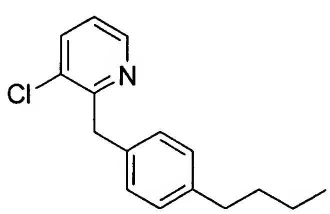


Potassium carbonate (33.0 mg, 0.239 mmol) and methyl iodide (14.9 μl, 0.239 mmol) were added to a solution of the compound of Example B230 (19.2 mg, 0.0796 mmol) in acetone (1 ml), and this reaction mixture was stirred at room temperature for 3 hours. After ethyl acetate was added, the reaction mixture was washed with saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (1.47 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.90(3H, t), 1.32-1.34(2H, m), 1.53-1.57(2H, m), 2.54(2H, t), 3.82(3H, s), 4.14(2H, s), 7.06(2H, d), 7.10-7.11(2H, m), 7.21(2H, d), 8.12-8.14(1H, m)

Example B232

2-(4-Butylbenzyl)-3-chloropyridine



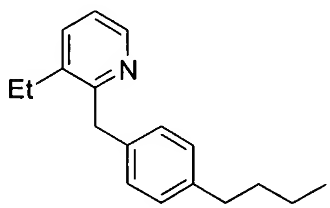
The compound of Example B205 (12 ml, 5 mmol) was added to an ice-cooled mixed solution of 2,3-dichloropyridine (525 mg, 3.55 mmol) and dichloro(diphenylphosphinopropane)nickel (96.2 mg, 0.178 mmol) in tetrahydrofuran (4 ml), and this reaction mixture was stirred at room temperature for 1 hour. After ethyl acetate was added, the reaction mixture was washed successively with a saturated aqueous ammonium

chloride solution, a saturated aqueous sodium hydrogencarbonate solution, and saturated brine, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (199 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.91(3H, t), 1.29-1.38(2H, m), 1.52-1.60(2H, m), 2.56(2H, t), 4.28(2H, s), 7.08-7.13(3H, m), 7.21(2H, d), 7.64(1H, dd), 8.46(1H, dd)

Example B233

2-(4-Butylbenzyl)-3-ethylpyridine

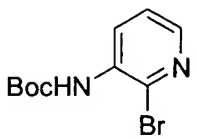


Ethylmagnesium chloride (0.97 M, 102 μl, 0.993 mmol) was added to a mixed solution of the compound of Example B232 (12.9 mg, 0.0496 mmol) and dichloro(diphenylphosphinoferrocene)nickel (3.4 mg, 0.0050 mmol) in tetrahydrofuran (1 ml). The reaction mixture was stirred at 50°C for 1 hour, then heated under reflux for another 2 hours. After allowing the reaction mixture to reach room temperature, ethyl acetate was added thereto. The reaction mixture was washed with a saturated aqueous ammonium chloride solution and saturated brine, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (3.29 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.90-0.93(6H, m), 1.30-1.37(2H, m), 1.54-1.59(2H, m), 2.55-2.59(4H, m), 4.12(2H, s), 7.05-7.18(5H, m), 7.55-7.59(1H, m), 8.53-8.55(1H, m)

Example B234

tert-Butyl *N*-(2-bromo-3-pyridyl)carbamate

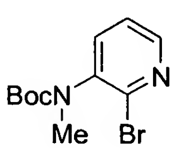


N-bromosuccinimide (7.51 g, 42.2 mmol) was added to an ice-cooled mixed solution of 3-aminopyridine (3.97 g, 42.2 mmol) in dimethylformamide (25 ml), and this reaction mixture was stirred at that temperature for 30 minutes. After ethyl acetate was added, the reaction mixture was washed with saturated brine and concentrated under reduced pressure. A solution of the residue in methylene chloride (20 ml) was cooled on ice, then triethylamine (3.74 ml, 26.8 mmol), a catalytic amount of dimethylaminopyridine, and di-*t*-butyl dicarbonate (3.08 ml, 13.4 mmol) were added to the solution, and the mixture was stirred at room temperature overnight. After concentration under reduced pressure, the residue was purified by silica gel column chromatography to give the title compound (344 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 1.55(9H, s), 7.03(1H, brs), 7.25(1H, dd), 8.03(1H, dd), 8.46(1H, d)

Example B235

2-Bromo-3-(*N*-*t*-butoxycarbonyl-*N*-methyl)aminopyridine

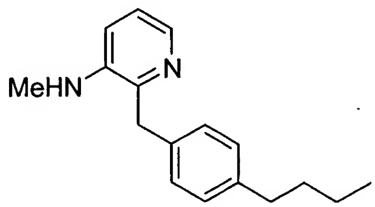


Methyl iodide (157 μl, 2.52 mmol) and 66% sodium hydride (91.6 mg, 2.52 mmol) were added to an ice-cooled solution of the compound of Example B234 (344 mg, 1.26 mmol) in dimethylformamide (5 ml), and this reaction mixture was stirred at that temperature for 40 minutes. After ethyl acetate was added, the reaction mixture was washed with saturated brine and filtered through silica gel. The organic layer was concentrated under reduced pressure to give the title compound (356 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 1.36(9H, s), 3.17(3H, s), 7.30(1H, dd), 7.55(1H, d), 8.30(1H, dd)

Example B236

N-[2-(4-Butylbenzyl)-3-pyridyl]-*N*-methylamine

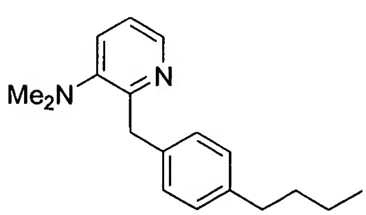


To a methylene chloride solution (2 ml) of a compound, which was obtained by introduction of a 4-butylbenzyl group to the compound of Example B235 (62.8 mg, 0.219 mmol) in the same manner as in Example B211, trifluoroacetic acid (2 ml) was added. The mixture was stirred at room temperature for 1 hour, and then added dropwise to an aqueous solution of sodium hydrogencarbonate. After ethyl acetate was added, the mixture was washed with saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (29.7 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.91(3H, t), 1.29-1.38(2H, m), 1.53-1.60(2H, m), 2.56(2H, t), 2.72(3H, s), 3.63(1H, br s), 4.09(2H, s), 6.86(1H, d), 7.08-7.12(5H, m), 7.98(1H, dd)

Example B237

N-[2-(4-Butylbenzyl)-3-pyridyl]-*N*,*N*-dimethylamine



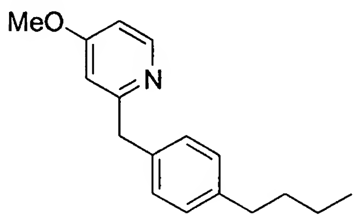
Acetic acid (12.1 μl, 0.211 mmol), 37% formalin (15.8 μl, 0.211 mmol), and sodium triacetoxyborohydride (44.7 mg, 0.211 mmol) were added to an ice-cooled solution of the compound of Example B236 (26.8 mg, 0.105 mmol) in methylene chloride (2 ml), and the mixture was stirred at room temperature for 30 minutes. After ethyl acetate was added, the mixture was washed with a saturated aqueous sodium hydrogencarbonate solution

and saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (23.3 mg)

^{1}H-NMR($CDCl_3$) δ (ppm): 0.91(3H, t), 1.30-1.36(2H, m), 1.52-1.59(2H, m), 2.55(2H, t), 2.67(6H, s), 4.24(2H, s), 7.06(2H, d), 7.10(1H, dd), 7.18(2H, d), 7.40(1H, dd), 8.27(1H, dd)

Example B238

2-(4-Butylbenzyl)-4-methoxypyridine

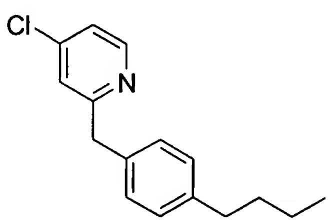


The title compound was obtained in the same manner as in Example B211 using 2-chloro-4-methoxypyridine.

^{1}H-NMR($CDCl_3$) δ (ppm): 0.91(3H, t), 1.31-1.37(2H, m), 1.53-1.59(2H, m), 2.57(2H, t), 3.78(3H, s), 4.06(2H, s), 6.61-6.65(2H, m), 7.11(2H, d), 7.17(2H, d), 8.36(1H, d)

Example B239

2-(4-Butylbenzyl)-4-chloropyridine



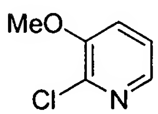
Phosphorus oxychloride (57.0 µl, 0.612 mmol) was added to an ice-cooled solution of the compound of Example B238 (52.0 mg, 0.204 mmol) in dimethylformamide (1 ml), and this reaction mixture was stirred at 100°C for 8 hours. The reaction mixture was allowed to cool, poured on ice, and warmed to room temperature. After ethyl acetate was added, the mixture was washed with a saturated aqueous sodium hydrogencarbonate

solution and saturated brine, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (2.29 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.92(3H, t), 1.31-1.38(2H, m), 1.53-1.61(2H, m), 2.59(2H, t), 4.10(2H, s), 7.12-.18(6H, m), 8.44(1H, d)

Example B240

2-Chloro-3-methoxypyridine

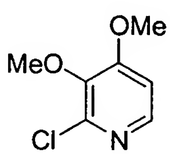


The title compound was obtained in the same manner as in Example B231 using 2-chloro-3-hydroxypyridine.

^{1}H-NMR($CDCl_3$) δ (ppm): 3.93(3H, s), 7.21-7.22(2H, m), 7.99-8.01(1H, m)

Example B241

2-Chloro-3,4-dimethoxypyridine



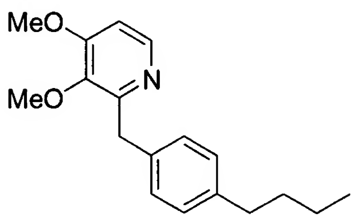
A solution of diisopropylamine (84.0 µl, 0.599 mmol) and the compound of Example B240 (860 mg, 5.99 mmol) in tetrahydrofuran (4 ml) was added to a solution of 1.06 M phenyllithium cyclopentane-diethyl ether solution in tetrahydrofuran (11 ml) cooled to −78°C under nitrogen atmosphere. This reaction mixture was stirred at −40°C for 1 hour, then at −18°C for another 20 minutes. The reaction mixture was cooled again to −78°C, trimethoxyborate (2.04 ml, 18.0 mmol) was added dropwise thereto, and the resulting mixture was stirred at 0°C for 20 minutes. At that temperature, aqueous ammonia (29%, 30 ml), ammonium chloride (4.5 g,), and an aqueous hydrogen peroxide solution (30%, 12 ml) were added in this order, and the mixture was stirred at room temperature for 2 hours. Saturated sodium thiosulfate, acetic acid and ethyl acetate were added, and the mixture was washed with saturated brine.

The ethyl acetate layer obtained upon filtration through silica gel was concentrated under reduced pressure. The resulting residue was treated in the same manner as in Example B231 to obtain the title compound (31.3 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 3.89(3H, s), 3.94(3H, s), 6.82(1H, d), 8.05(1H, d)

Example B242

2-(4-Butylbenzyl)-3,4-dimethoxypyridine

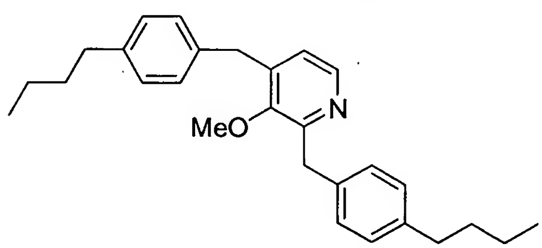


The title compound was obtained in the same manner as in Example B206 using the compound of Example B241.

^{1}H-NMR($CDCl_3$) δ (ppm): 0.90(3H, t), 1.26-1.35(2H, m), 1.53-1.57(2H, m), 2.54(2H, t), 3.70(3H, s), 3.89(3H, s), 4.12(2H, s), 6.72(1H, d), 7.06(2H, d), 7.21(2H, d), 8.20(1H, d)

Example B243

2,4-Di-(4-butylbenzyl)-3-methoxypyridine



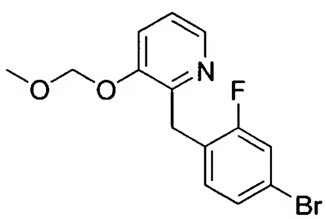
A solution of the compound of Example B240 (436 mg, 3.04 mmol) in diethyl ether (2 ml) was added to a solution of 1.43 M *t*-butyllithium *n*-pentane solution (2.76 ml, 3.95 mmol) in diethyl ether (5 ml) cooled to -78°C under nitrogen atmosphere, and this reaction mixture was stirred at that temperature for 30 minutes. A solution of tetramethylethylenediamine (688 µl, 4.56 mmol) and hexachloroethane

(719 mg, 3.04 mmol) in diethyl ether (3 ml) was further added and the reaction mixture was stirred at that temperature for 1 hour. After warming gradually to room temperature, ethyl acetate was added, and the mixture was washed with saturated brine. The ethyl acetate layer obtained upon filtration through silica gel was concentrated under reduced pressure. The resulting residue was treated in the same manner as in Example B206 to obtain the title compound (10.1 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 0.89-0.94(6H, m), 1.31-1.37(4H, m), 1.52-1.62(4H, m), 2.53-2.59(4H, m), 3.74(3H, s), 4.07(2H, s), 4.13(2H, s), 6.84(1H, d), 6.98(1H, d), 7.04-7.22(8H, m)

Example B244

2-(4-Bromo-2-fluorobenzyl)-3-(methoxymethoxy)pyridine

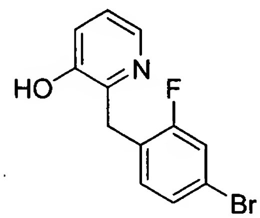


A solution of the compound of Example B228 (422 mg, 1.94 mmol) in tetrahydrofuran (3 ml) was added to a solution of 2.47 M *n*-butyllithium *n*-hexane solution (862 μl, 2.13 mmol) in tetrahydrofuran (3 ml) cooled to −78°C under nitrogen atmosphere, and this reaction mixture was stirred at that temperature for 1 hour. After copper(I) bromide (139 mg, 0.968 mmol) was added, the reaction mixture was stirred at 0°C for 1 hour and cooled again to −78°C. Next, 4-bromo-2-fluorobenzyl bromide (259 mg, 0.968 mmol) was added, and the resulting mixture was stirred at 0°C for 1 hour. Tetramethylethylenediamine (584 μl, 3.88 mmol) was further added, and the resulting reaction mixture was stirred at that temperature for 1 hour. After diethyl ether and an aqueous ammonia solution were added to the reaction mixture, the organic layer was washed with saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (81.0 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 3.38(3H, s), 4.17(2H, s), 5.18(2H, s), 7.04(1H, t), 7.11-7.22(3H, m), 7.38(1H, dd), 8.19(1H, dd)

Example B245

2-(4-Bromo-2-fluorobenzyl)-3-pyridinol



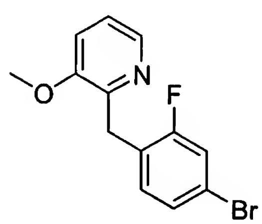
Trifluoroacetic acid (1 ml) was added to the compound of Example B244 (134 mg, 0.411 mmol) in methylene chloride (4 ml), and this reaction mixture was stirred at room temperature overnight. After neutralizing the mixture with saturated aqueous sodium hydrogencarbonate, ethyl acetate was added. The ethyl acetate layer was washed with saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (97.5 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 4.17(2H, s), 7.10-7.24(5H, m), 8.15(1H, t)

The proton of the phenolic hydroxyl group was not observed in the NMR spectrum.

Example B246

2-(4-Bromo-2-fluorobenzyl)-3-methoxypyridine



Potassium carbonate (38.7 mg, 0.280 mmol) and methyl iodide (10.5 μl, 0.168 mmol) were added to a solution of the compound of Example B245 (15.8 mg, 0.0560 mmol) in dimethylformamide (1 ml), and this reaction mixture was stirred at room temperature for 2 hours. After ethyl acetate

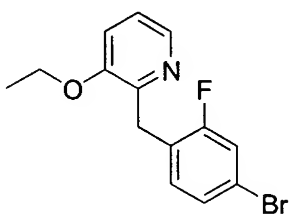
was added, the reaction mixture was washed with saturated brine and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the title compound (14.0 mg).

^{1}H-NMR($CDCl_3$) δ (ppm): 3.82(3H, s), 4.15(2H, s), 7.03(1H, t), 7.12-7.22(4H, m), 8.13(1H, dd)

The following compounds of Example B were synthesized in the same manner as in Example B246, and purification was performed by LC-MS [eluent: an acetonitrile solution containing 0.1% trifluoroacetic acid: an aqueous solution containing 0.1% trifluoroacetic acid = 1:99 to 100:0/20-minute cycle, flow rate: 20 ml/minute, column: YMC Combiprep ODS-AM, 20 mm Φx 50 mm (long)].

Example B247

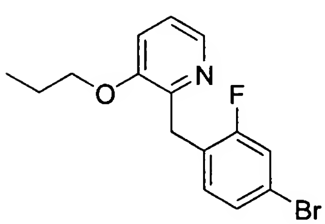
2-(4-Bromo-2-fluorobenzyl)-3-ethoxypyridine



MS m/z (ESI: MH$^+$): 310.0

Example B248

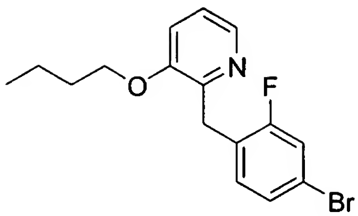
2-(4-Bromo-2-fluorobenzyl)-3-propoxypyridine



MS m/z (ESI: MH$^+$): 324.0

Example B249

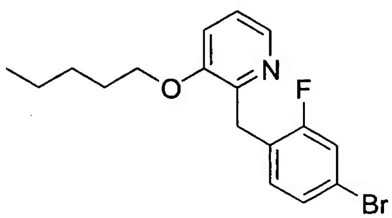
2-(4-Bromo-2-fluorobenzyl)-3-butoxypyridine



MS m/z (ESI: MH^+): 338.1

Example B250

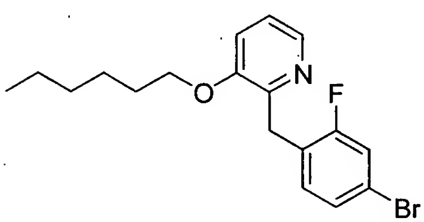
2-(4-Bromo-2-fluorobenzyl)-3-(pentyloxy)pyridine



MS m/z (ESI: MH^+): 352.1

Example B251

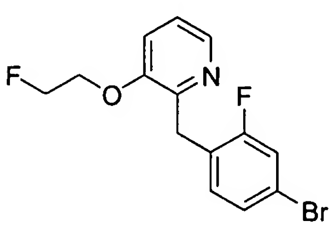
2-(4-Bromo-2-fluorobenzyl)-3-(hexyloxy)pyridine



MS m/z (ESI: MH^+): 366.0

Example B252

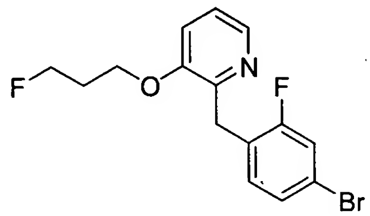
2-(4-Bromo-2-fluorobenzyl)-3-(2-fluoroethoxy)pyridine



MS m/z (ESI: MH^+): 328.0

Example B253

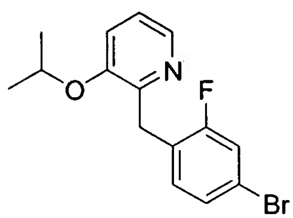
2-(4-Bromo-2-fluorobenzyl)-3-(3-fluoropropoxy)pyridine



MS m/z (ESI: MH^+): 342.0

Example B254

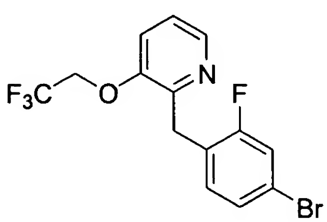
2-(4-Bromo-2-fluorobenzyl)-3-isopropoxypyridine



MS m/z (ESI: MH^+): 324.0

Example B255

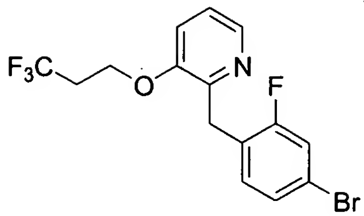
2-(4-Bromo-2-fluorobenzyl)-3-(2,2,2-trifluoroethoxy)pyridine



MS m/z (ESI: MH^+): 364.0

Example B256

2-(4-Bromo-2-fluorobenzyl)-3-(3,3,3-trifluoropropoxy)pyridine



MS m/z (ESI: MH^+): 378.0

Example B257

Compounds were evaluated using the *S. cerevisiae* reporter system of Example A2. The lowest concentration at which cephalosporinase activity in the cell wall fraction became 50% or less compared to that obtained where the compound was not treated, was defined to be the IC50 value. Effects of the representative compounds are shown in Table 1.

Table 1

Compound	IC50 (μg/ml)
1-(4-butylbenzyl)isoquinoline (Example B2)	0.39
*N*1-{3-[4-(1-isoquinolylmethyl)phenyl]-2-propynyl} acetamide (Example B60)	6.25
*N*1-{3-[4-(1-isoquinolylmethyl)phenyl]propyl}-*N*1-methyl acetamide (Example B73)	50
5-butyl-2-(1-isoquinolylmethyl)phenol (Example B85)	0.20
4-(4-butylbenzyl)thieno[3,2-*c*]pyridine (Example B187)	0.78
7-(4-butylbenzyl)thieno[2,3-*c*]pyridine (Example B195)	0.39
2-(4-butylbenzyl)-3-methoxypyridine (Example B231)	0.78
2-(4-butylbenzyl)-3,4-dimethoxypyridine (Example B242)	0.78

Industrial Applicability

The present invention revealed genes encoding the proteins participating in the transport process of the GPI-anchored proteins to the cell wall. Furthermore, this invention discloses a method of

screening for compounds that inhibit the activity of these proteins, and also discloses representative compounds having the inhibitory activity.

Using novel compounds, the present invention showed that antifungal agents having a novel mechanism of inhibiting the transport process of the GPI-anchored proteins to the cell wall can be provided.

CLAIMS

1. A DNA that encodes a protein having an activity to confer resistance of a fungus against the compound shown in formula (Ia) when the DNA is overexpressed in the fungus, wherein the DNA is selected from the group consisting of:

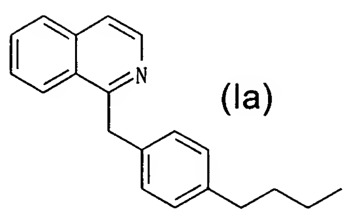
(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59,

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58,

(c) a DNA that hybridizes under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58,

(d) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59, wherein one or more amino acids have been added, deleted, substituted, and/or inserted, and

(e) a DNA that is amplified using SEQ ID NOS: 29 and 31 or SEQ ID NOS: 29 and 30 as primers



(Ia)

2. A DNA that encodes a protein having an activity to decrease the amount of a GPI-anchored protein in the cell wall of a fungus due to a defect in the function of the DNA, wherein the DNA is selected from the group consisting of:

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59,

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58,

(c) a DNA that hybridizes under stringent conditions to a DNA

comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 27, 39, 41, 54, or 58,

(d) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 28, 40, or 59, wherein one or more amino acids have been added, deleted, substituted, and/or inserted, and

(e) a DNA that is amplified using SEQ ID NOS: 29 and 31 or SEQ ID NOS: 29 and 30 as primers.

3. A protein encoded by the DNA of claim 1 or 2.

4. A vector into which the DNA of claim 1 or 2 has been inserted.

5. A transformant harboring the DNA of claim 1 or 2, or the vector of claim 4.

6. The transformant of claim 5 which is a fungus that overexpresses the protein of claim 3.

7. A fungus, wherein the function of the protein of claim 3 is defective.

8. A method for producing the protein of claim 3, which comprises the steps of culturing the transformant of claim 5, and collecting the expressed protein from the transformant, or from the culture supernatant thereof.

9. An antibody that binds to the protein of claim 3.

10. A method of screening for a compound having an antifungal action, wherein the method comprises the steps of:

(a) contacting a test sample with the protein of claim 3;

(b) detecting the binding activity between the protein and the test sample; and

(c) selecting a compound having an activity to bind to the protein.

11. A method of screening for a compound that has an antifungal action, which comprises the steps of:

(a) contacting a test sample with a fungus that is overexpressing the protein of claim 3;

(b) detecting the amount of transport of a GPI-anchored protein to the cell wall in the fungus; and

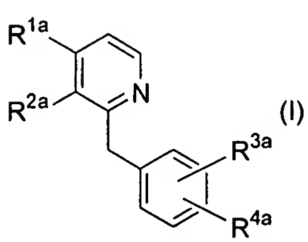
(c) selecting a compound that diminishes the amount of transport of the GPI-anchored protein to the cell wall detected in step (b) as compared to the amount of transport detected when the test sample was contacted with a fungus that is not overexpressing the protein of claim 3.

12. A compound having an antifungal action that is isolated by the screening of claim 10 or 11.

13. An antifungal agent, comprising as an active ingredient a compound that inhibits the transport of GPI-anchored proteins to the cell wall of a fungus.

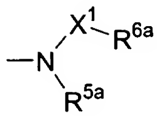
14. An antifungal agent, comprising as an active ingredient the antibody of claim 9 or the compound of claim 12.

15. The antifungal agent of claim 13, comprising as an active ingredient the compound represented by the general formula (I), a salt thereof, or a hydrate thereof, wherein in formula (I):



[R^{1a} and R^{2a} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro

group, cyano group, trifluoromethyl group, trifluoromethoxy group, a substituted or unsubstituted C_{1-6} alkyl group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, a substituted or unsubstituted C_{1-6} alkoxy group, or a group represented by the formula:

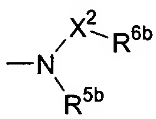


(wherein X^1 stands for a single bond, carbonyl group, or a group represented by the formula $-S(O)_2-$;

R^{5a} and R^{6a} are identical to or different from each other and denote a hydrogen atom or a substituted or unsubstituted C_{1-6} alkyl group); R^{1a} and R^{2a} may form together a condensed ring selected from the group consisting of a substituted or unsubstituted benzene ring, a substituted or unsubstituted pyridine ring, a substituted or unsubstituted pyrrole ring, a substituted or unsubstituted thiophene ring, a substituted or unsubstituted furan ring, a substituted or unsubstituted pyridazine ring, a substituted or unsubstituted pyrimidine ring, a substituted or unsubstituted pyrazine ring, a substituted or unsubstituted imidazole ring, a substituted or unsubstituted oxazole ring, a substituted or unsubstituted thiazole ring, a substituted or unsubstituted pyrazole ring, a substituted or unsubstituted isoxazole ring, a substituted or unsubstituted isothiazole ring, a substituted or unsubstituted cyclohexane ring, and a substituted or unsubstituted cyclopentane ring;

R^{3a} and R^{4a} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro group, cyano group, carboxyl group, formyl group, hydroxyimino group, trifluoromethyl group, trifluoromethoxy group, C_{1-6} alkyl group, C_{1-6} alkoxy group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, a group represented by the formula $-C(O)NR^{7a}R^{7b}$ (wherein R^{7a} and

R^{7b} are identical to or different from each other and denote individually a hydrogen atom, or a C_{1-6} alkyl group), the formula $-CO_2R^{7a}$ (wherein R^{7a} has the same meaning as defined above), the formula $-S(O)_nR^{7a}$ (wherein n stands for an integer of 0 to 2 and R^{7a} has the same meaning as defined above), the formula $-S(O)_2NR^{7a}R^{7b}$ (wherein R^{7a} and R^{7b} have the same meaning as defined above), a group of the formula



(wherein X^2 denotes a single bond, carbonyl group, or a group of the formula $-S(O)_2-$;

R^{5b} and R^{6b} are identical to or different from each other, and denote a hydrogen atom, a substituted or unsubstituted C_{1-6} alkyl group, or a substituted or unsubstituted C_{6-14} aryl group), or a group of the formula

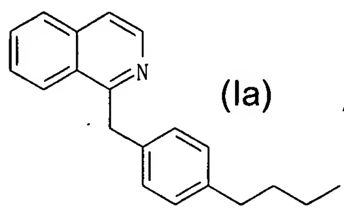
$$-Z^1-Z^2$$

(wherein Z^1 denotes a single bond, oxygen atom, vinylene group, or ethynylene group;

Z^2 denotes a single bond, or a C_{1-6} alkyl group substituted or unsubstituted with 0 to 4 substituents); R^{3a} and R^{4a} may together stand for a methylenedioxy group or 1,2-ethylenedioxy group, alternatively, R^{3a} and R^{4a} may together stand for the formation of a condensed ring selected from a group consisting of a substituted or unsubstituted benzene ring, substituted or unsubstituted pyridine ring, substituted or unsubstituted pyrrole ring, substituted or unsubstituted thiophene ring, substituted or unsubstituted furan ring, substituted or unsubstituted pyridazine ring, substituted or unsubstituted pyrimidine ring, substituted or unsubstituted pyrazine ring, substituted or unsubstituted imidazole ring, substituted or

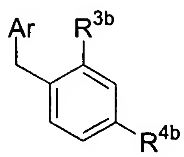
unsubstituted oxazole ring, substituted or unsubstituted thiazole ring, substituted or unsubstituted pyrazole ring, substituted or unsubstituted isoxazole ring, substituted or unsubstituted isothiazole ring, substituted or unsubstituted cyclohexane ring, and substituted or unsubstituted cyclopentane ring, except in cases where both R^{1a} and R^{2a} do not stand for hydrogen atoms].

16. The antifungal agent of claim 13, comprising as the active ingredient compound (Ia) of the formula:



(Ia) ,

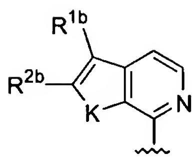
17. A compound represented by the formula (II), a salt or a hydrate thereof, wherein in formula (II),



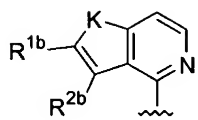
(II)

[Ar stands for a substituent selected from a group consisting of the formulae (IIIa) to (IIIf):

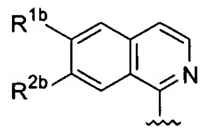
(IIIa)



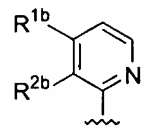
(IIIb)



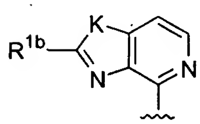
(IIIc)



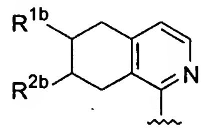
(IIId)



(IIIe)

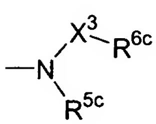


(IIIf)



(wherein K denotes a sulfur atom, oxygen atom, or a group represented by the formula -NH-;

R^{1b} and R^{2b} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro group, cyano group, trifluoromethyl group, trifluoromethoxy group, a group represented by the formula



(wherein X^3 denotes a single bond, carbonyl group, or a group represented by the formula $-S(O)_2-$;

R^{5c} and R^{6c} are identical to or different from each other and denote a hydrogen atom, or a substituted or unsubstituted C_{1-6} alkyl group), or a group represented by the formula $-X^4-R^{8a}$ (wherein X^4 denotes a single bond, oxygen atom, or sulfur atom; R^{8a} denotes a C_{1-6} alkyl group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, C_{3-8} cycloalkyl group, or C_{3-8} cycloalkenyl group); R^{1b} and R^{2b} together may form a methylenedioxy group, or a 1,2-ethylenedioxy group);

R^{3b} and R^{4b} are identical to or different from each other and denote individually a hydrogen atom, halogen atom, hydroxyl group, nitro

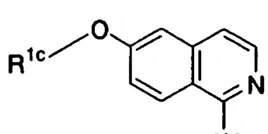
group, cyano group, carboxyl group, formyl group, hydroxyimino group, trifluoromethyl group, trifluoromethoxy group, C_{1-6} alkyl group, C_{1-6} alkoxy group, C_{2-6} alkenyl group, C_{2-6} alkynyl group, or a group represented by the formula

$$—Z^{1b}–Z^{2b}$$

(wherein Z^{1b} denotes a single bond, vinylene group, or ethynylene group;

Z^{2b} denotes a single bond, or a C_{1-6} alkyl group that is substituted or unsubstituted with 0 to 4 substituents);
except in cases where (1) Ar stands for the aforementioned formula (IIId) wherein R^{1b} and R^{2b} are both hydrogen atoms, (2) at least one of R^{3b} or R^{4b} denotes a hydrogen atom and the other is a hydrogen atom, methoxy group, hydroxyl group, methyl group, benzyloxy group, or a halogen atom, and Ar stands for the aforementioned formula (IIIc) wherein R^{1b} and R^{2b} both denote hydrogen atoms or methoxy groups, (3) at least one of R^{3b} or R^{4b} denotes a hydrogen atom and the other is a hydrogen atom, hydroxyl group, methoxy group, or benzyloxy group, and Ar stands for the formula (IIIc) wherein R^{1b} and R^{2b} both denote hydroxyl groups or benzyloxy groups, or (4) Ar stands for the formula (IIId) wherein R^{1b} is a hydrogen atom and R^{2b} is a formyl group, hydroxymethyl group, or methoxycarbonyl group].

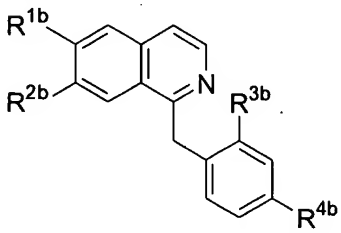
18. The compound of claim 17, or a salt or hydrate thereof, wherein Ar stands for the formula:



(IIIg)

(wherein R^{1c} denotes a hydrogen atom, a substituted or unsubstituted C_{1-6} alkyl group, or a benzyl group), and excluding the case when R^{3b} denotes a hydrogen atom.

19. A compound represented by the formula (IIIc2), or a salt or hydrate thereof, wherein in formula (IIIc2),

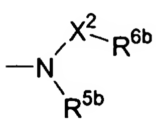


(IIIc2)

[R^{1b} and R^{2b} have the same meaning as defined above, except in cases wherein (1) R^{1b} denotes a group represented by the formula R^{1c}-O- (wherein R^{1c} has the same meaning as defined above), R^{2b} is a hydrogen atom, and R^{3b} denotes a hydrogen atom, (2) at least one of R^{3b} or R^{4b} denotes a hydrogen atom, and the other is a hydrogen atom, methoxy group, hydroxyl group, methyl group, benzyloxy group, or a halogen atom, and R^{1b} and R^{2b} both denote hydrogen atoms or methoxy groups, or (3) at least one of R^{3b} or R^{4b} denotes a hydrogen atom, and the other is a hydrogen atom, hydroxyl group, methoxy group, or benzyloxy group, and R^{1b} and R^{2b} both denote hydroxyl groups or benzyloxy groups].

20. The antifungal agent of claim 17, having an antifungal action.

21. The antifungal agent of claim 15, wherein at least one of R^{3a} and R^{4a} denotes a group represented by the formula $-C(O)NR^{7a}R^{7b}$ (wherein R^{7a} and R^{7b} have the same meaning as defined above), the formula $-CO_2R^{7a}$ (wherein R^{7a} has the same meaning as defined above), the formula $-S(O)_nR^{7a}$ (wherein n denotes an integer of 0 to 2 and R^{7a} has the same meaning as defined above), the formula $-S(O)_2NR^{7a}R^{7b}$ (wherein R^{7a} and R^{7b} have the same meaning as defined above), the formula



(wherein X^2, R^{5b}, and R^{6b} have the same meaning as defined above), or

a C_{1-6} alkoxy group substituted or unsubstituted with 0 to 4 substituents, or R^{3a} and R^{4a} together denote a methylenedioxy group, or a 1,2-ethylenedioxy group.

22. The antifungal agent of claim 15, wherein the compound having an antifungal action is (1) 1-benzylisoquinoline, (2) 1-(4-bromobenzyl)isoquinoline, (3) 1-(4-chlorobenzyl)isoquinoline, (4) 1-(4-fluorobenzyl)isoquinoline, (5) 1-(4-iodobenzyl)isoquinoline, (6) 1-(3-methylbenzyl)isoquinoline, (7) 1-(4-methylbenzyl)isoquinoline, (8) 1-(3,4-dimethylbenzyl)isoquinoline, (9) 1-(3-methoxybenzyl)isoquinoline, (10) 1-(4-methoxybenzyl)isoquinoline, (11) 1-(3,4-methylenedioxybenzyl)isoquinoline, (12) 1-(4-benzyloxybenzyl)isoquinoline, (13) 1-(4-cyanobenzyl)isoquinoline, (14) 1-(4-nitrobenzyl)isoquinoline, (15) 1-(4-aminobenzyl)isoquinoline, (16) 1-(4-methoxybenzyl)-6,7-dichloro-isoquinoline, (17) 1-(4-methoxy-2-nitro-benzyl)-isoquinoline, (18) 1-(4-methoxybenzyl)-6,7-methylenedioxy-isoquinoline, (19) 1-(2-amino-4-methoxy-benzyl)isoquinoline, (20) 1-(4-methoxybenzyl)-7-hydroxy-6-methoxy-isoquinoline, (21) 1-(4-benzyloxybenzyl)-6,7-dimethoxy-isoquinoline, (22) 1-(4-methoxybenzyl)-6,7-dimethoxy-isoquinoline, (23) 1(4-methoxy-2-nitro-benzyl)-isoquinoline, (24) 3-[4-(1-isoquinolylmethyl)phenoxy]propylcyanide, (25) 1-[4-(2,2,3,3-tetrafluoropropoxy)benzyl]isoquinoline, (26) 1-[4-(2-piperidinoethoxy)benzyl]isoquinoline, (27) 4-(1-isoquinolylmethyl)phenyl(2-morpholinoethyl)ether, (28) 1-[4-(2-methoxyethoxy)benzyl]isoquinoline, (29) *N*-{2-[4-(1-isoquinolylmethyl)phenoxy]ethyl}-*N*,*N*-dimethylamine, (30) 1-[4-(phenethyloxy)benzyl]isoquinoline, (31) 1-{4-[(2-methylallyl)oxy]benzyl}isoquinoline, (32)

1-(4-isobutoxybenzyl)isoquinoline, (33) 1-[4-(2-phenoxyethoxy)benzyl]isoquinoline, (34) methyl 2-[4-(1-isoquinolylmethyl)phenoxy]acetate, (35) 2-[4-(1-isoquinolylmethyl)phenoxy]-1-ethanol, (36) t-butyl *N*-{2-[4-(1-isoquinolylmethyl)phenoxy]ethyl}carbamate, (37) 1-{4-[3-(tetrahydro-2H-2-pyranyloxy)propoxy]benzyl}isoquinoline, (38) 2-[4-(1-isoquinolylmethyl)phenoxy]-1-ethaneamine, (39) 1-[4-(3-piperidinopropoxy)benzyl]isoquinoline, (40) 3-[4-(1-isoquinolylmethyl)phenoxy]-1-propanol, (41) 1-[4-(2-ethylbutoxy)benzyl]isoquinoline, (42) 4-[4-(1-isoquinolylmethyl)phenoxy]butanoic acid, (43) 1-(4-{3-[(4-benzylpiperazino)sulfonyl]propoxy}benzyl)isoquinoline, (44) 1-(4-{3-[4-(4-chlorophenyl)piperazino]propoxy}benzyl)isoquinoline, (45) 4-(1-isoquinolylmethyl)aniline, (46) *N*-[4-(1-isoquinolylmethyl)phenyl]butaneamide, (47) *N*-[4-(1-isoquinolylmethyl)phenyl]propaneamide, (48) *N*-[4-(1-isoquinolylmethyl)phenyl]-1-ethanesulfonamide, (49) *N*-[4-(1-isoquinolylmethyl)phenyl]-*N*-methyl-ethanesulfonamide, (50) *N*-[4-(1-isoquinolylmethyl)phenyl]-*N*-methylamine, (51) *N*-[4-(1-isoquinolylmethyl)phenyl]-*N*-propylamine, or (52) *N*-[4-(1-isoquinolylmethyl)phenyl]-*N*-methyl-*N*-propylamine.

23. A method for treating a mycotic infection comprising administering a therapeutically effective dose of any one of the antifungal agents of claims 13 to 22 to a mammal.

ABSTRACT

A reporter system reflecting the transport process that transports GPI-anchored proteins to the cell wall was constructed and compounds inhibiting this process were discovered. Further, genes conferring resistance to the above compounds were identified and methods of screening for compounds that inhibit the activity of the proteins encoded by these genes were developed.

Therefore, through the novel compounds, the present invention showed that antifungal agents having a novel mechanism, i.e. inhibiting the process that transports GPI-anchored proteins to the cell wall, could be achieved.